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THE
ANATOMICAL RECORD

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THE PERIVASCULAR SPACES OF THE MAMMALIAN BRAIN[1]

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Department of Anatomy, The University of Southern California, School of Medicine, Los Angeles

SIX PLATES (NINETEEN FIGURES)

INTRODUCTION

Although the perivascular spaces of the brain have been studied, the knowledge derived from these studies is incomplete and confused, because the methods used did not permit accurate distinction between fact and artefact.

The first account of the perivascular spaces, according to Schaltenbrand and Bailey ('28), was given by Pestalozzi in 1849 in his description of hemorrhage into the walls of the vessels of the brain. This hemorrhage was described as being between the adventitia and media of the larger vessels. Vesicular swellings were also seen at the branchings of smaller vessels. These findings were confirmed by Virchow in 1851 who observed spaces between the adventitia and media of the vessels and at times around capillaries. These spaces, he said, may be dilated by a fluid or different types of cells. In 1859 Robin reported perivascular spaces as being normally existing structures. These he thought to be closed and to lie within the adventitia proper and not between adventitia and media.

By means of an intracerebral multiple puncture type of injection, His (1865) filled spaces which he described as appearing at first like artefacts. These he called lymphatics

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[1] The author is sincerely grateful to the late Prof. Paul S. McKibben and to Prof. D. B. MacCallum for their many helpful suggestions. Appreciation is also extended to Mr. A. B. Streedain for his aid in the preparation of the illustrations.

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since they were surrounded by endothelial-like cells and were continuous with lymphatic-like structures which he believed to be located between the brain tissue and the surface pia. His thought the perivascular lymphatics to be between the blood vessels and the brain substance and to possess expansions which surrounded nerve cells. These latter structures, although seen, were not injected.

Key and Retzius (1876) reported the finding of endothelial lined perivascular spaces extending well into the brain substance. Visceral and parietal layers continuous with the linings of the subarachnoid space were differentiated. Their study of astrocytes and their processes revealed glial feet attaching directly to the capillary adventitia and to the glial membrane surrounding the larger vessels. Key and Retzius were the first to believe that the perivascular lymphatics described by His were artefacts and considered them to lie between the true perivascular space and the glial membrane.

A potential space lying between the glial membrane and the brain substance was described in 1909 by Held. He differentiated this space from the true perivascular space which he believed to have been described correctly by Key and Retzius. In this study of the glial membrane Held stained the tissues with hematoxylin and concluded that the glial fibers formed a syncytium. This finding, however, was disputed by Achucarro ('13) and others who used silver impregnation techniques. Tuke (1894) and Mott ('10) described and illustrated perivascular spaces which were almost identical with those described by His. Bruce and Dawson ('20), like Virchow, reported adventitial spaces surrounding the vessels of the brain. They compared their findings to those of His and concluded that the spaces described by His must be artefacts.

Weed and McKibben ('19) and Weed ('23) attempted to demonstrate the perivascular spaces by injecting a hypertonic solution of sodium chloride intravenously in an anaesthetized animal. At the same time they injected an isotonic solution of potassium ferrocyanide and iron-ammonium citrate into the subarachnoid space. The animal was then killed and the brain was immediately fixed by a vascular perfusion with acid formalin. The sodium chloride solution increased the osmotic pressure of the blood, thus withdrawing fluid from the brain. Cerebro-spinal fluid containing potassium ferrocyanide and iron-ammonium citrate was therefore drawn from the subarachnoid space towards the capillaries through perivascular spaces. The potassium ferrocyanide and iron-ammonium citrate were precipitated as Prussian blue granules by the acid formalin. Examination of the brain revealed Prussian blue granules within perivascular spaces surrounding arteries, veins, and capillaries. Granules were also located in the pericellular spaces, around glial cells, and amongst the white fibers and supporting elements of the brain. Weed also substituted India ink for the true solution of potassium ferrocyanide and iron-ammonium citrate. In these experiments the India ink particles only incompletely filled perivascular or other spaces. No detailed study of the cellular structure of the spaces injected was reported.

Schaltenbrand and Bailey ('27) reviewed and discussed the extensive literature on the perivascular spaces. Their description of the spaces, however, was derived essentially from pathological material.

MATERIALS AND METHODS

To demonstrate the true perivascular spaces, their cellular structure, and at the same time to differentiate them from potential spaces or artefacts, a modification of the method used by Weed and McKibben ('19) and by Weed ('23) was found most suitable. Briefly, the method of Weed and McKibben involved dehydration of the brain and cord of the living animal by increasing the osmotic pressure of the blood, thus permitting potassium ferrocyanide and iron-ammonium citrate injected into the subarachnoid space, to be withdrawn from that space into the perivascular space, in a direction opposite to the normal flow of the cerebro-spinal fluid. The potassium ferrocyanide and iron-ammonium citrate were precipitated as Prussian blue by fixing the tissues in acid formalin.

The materials used for subarachnoid injections in this series of experiments were India ink or colloidal mercury sulfide, while the dehydrating agent, as in Weed's experiments, was a hypertonic solution of sodium chloride.

Twenty-four animals were used in all, twelve rabbits, six cats and six dogs. Eleven of these animals were anesthetized with nembutal or ether. A hypodermic needle was inserted into the cisterna magna and another into the femoral vein. Thirty per cent sodium chloride was slowly injected intravenously and at the same time either India ink or colloidal mercury sulfide was allowed to enter the subarachnoid space. In the rabbit and cat experiments 6 to 8 cc. of salt solution and 3 or 4 cc. of the particulate suspension were injected. In the dog experiments 8 to 10 cc. of the salt solution and 5 to 8 cc. of the particulate suspension were injected. The intravenous injections required about 10 minutes. The subarachnoid injections under atmospheric pressure lasted 15 to 20 minutes. Eighty minutes following the start of the procedure the animals were killed by bleeding. The blood vessels of the brain and spinal cord were perfused in situ through the aorta with 10% formalin; the brain and cord were then removed and fixation was continued by immersion in 10% formalin.

Sections 1 mm. in thickness were cut, dehydrated, cleared, and mounted unstained. Sections 10 to 50 micra thick were prepared by the paraffin or celloidin embedding methods. The stains used were Einarson's gallocyanin, Nissl's stain, hematoxylin, and a number of the silver impregnations. Del Rio-Hortega's ('17) silver carbonate (lithium) proved to be particularly suitable.

Thirteen animals were sacrificed as controls. Two received the subarachnoid injection under pressure of the particulate suspension alone; while two received only the intravenous injection of hypertonic salt solution. One animal received no injections. The procedures of Bielschowsky and Cobb ('25) to demonstrate the lining cells of the perivascular spaces were repeated in two animals; the multiple puncture injection method of His was repeated in three animals, and the method of Weed and McKibben was repeated in another similar group.

RESULTS

Gross findings

The brains of the injected animals are for the most part slightly smaller than normal, the dehydration by the intravenous hypertonic salt solution having caused some shrinkage. This shrinkage is not marked. The brain substance instead of bulging slightly through a trephined opening in the skull is flush with the inner edge of the bony orifice. In the control animals receiving only intravenous hypertonic salt solution, the brains are considerably below the trephined opening indicating shrinkage, which is in accordance with the findings of Weed and McKibben ('18). The subarachnoid injection therefore prevents marked alteration in brain bulk.

The blood vessels of the meninges are surrounded and outlined by particulate material drawn into the subarachnoid space and the perivascular space apertures surrounding the entrance of blood vessels into the brain substance are well filled with this foreign material. The controls which received only the subarachnoid injection under pressure exhibit none of these findings.

Thick sections

Thick, 1 mm. unstained sections show perivascular spaces filled and outlined by India ink or colloidal mercury sulphide. The India ink penetrates the perivascular spaces as far as the arteries and venules while the colloidal mercury sulphide could be found in the region surrounding the capillaries. The superficial appearance of the sections is similar to a vascular perfusion; but close observation discloses no foreign material in the blood vessels (figs. 1, 2, 3 and 4).

Cellular membranes

Recognition of the cellular membranes lining the perivascular spaces was facilitated by the injected materials plus the staining of their nuclei, the nuclei of nerve cells, and the nuclei and processes of neural connective tissue cells. Since the perivascular space may be bounded by two mesothelial cell membranes, an inner or vascular layer and an outer or neural layer, they were differentiated by observing whether the mesothelial cell nuclei were deep or superficial to the injected substance. The Bielschowsky and Cobb method of staining these membranes was unsatisfactory.

Lining the inner wall of the perivascular space (fig. 14) is a single mesothelial cell layer continuous with the arachnoid. This membrane is well defined by uniformly spaced nuclei. It is easily demonstrated accompanying both arteries and veins as far as the arterioles and venules and forms a complete membrane overlying the rather thin adventia of the blood vessels. The capillaries (fig. 11) possess no continuous covering membrane and like those of other tissues, they possess only occasional fibrocytes and pericapillary histiocytes.

The outer membrane (fig. 19) surrounding the perivascular spaces is continuous with the pia. Like the inner lining of the perivascular spaces, it is also composed of a single layer of mesothelial cells. This membrane accompanies the arteries for only a very short distance, varying from 1 to 2 mm. (fig. 5). As the termination of this perivascular extension of the pia is approached, the nuclei become less uniformly arranged and are spaced farther and farther apart, finally disappearing completely. The irregular arrangement and spacing of the nuclei suggest a terminal fenestrated membrane. Beyond this fenestrated membrane there are no nuclei peripheral to the injected substance, except for the neural connective tissue cell nuclei (fig. 7). Accompanying the veins, however, this mesothelial cell layer extends as far as the venules before it disappears (fig. 19). Its termination has the same characteristic appearance as the outer lining surrounding the perivascular spaces of the arteries. In four animals the macrophages of the pia and arachnoid membrane lining the subarachnoid space and their perivascular extensions, contained phagocytized colloidal mercury sulphide (fig. 9). This finding is similar to that of Zylberblast-Zand ('24) who demonstrated macrophages in the lining cells of the perivascular space following intravenous injections of trypan blue.

Glial membrane

Attaching directly to the endothelial cell wall of the capillaries are the processes of astrocytes, both fibrous and protoplasmic (fig. 15). These processes are fixed directly to the endothelial cells, no mesothelial cell layers intervening. Such fibers may be connected to the capillaries by true perivascular feet, branching fibre tufts, or directly by undifferentiated endings. Occasionally the fibers pass along the capillary surface for short distances, but they do not form any membrane or sheath. Perivascular astrocytes of Andriezen (1893) were occasionally observed, their elongated cell bodies resting on the capillary and their processes accompanying the vessels or passing into the surrounding tissue. Also seen were perivascular oligodendrocytes and microglia with processes surrounding or extending along the capillary wall. No pericapillary structure which might conceivably be called a glial membrane was noted.

The processes of astrocytes in the vicinity of the arterioles and venules do not attach to the vessel walls but accompany the vessels, intermingling and forming a loose mesh network of fibers separated by large spaces (fig. 16). This meshwork appears to be the central beginning of a glial membrane. Since there is no outer mesothelial cell wall surrounding the perivascular spaces of arterioles and precapillary parts of venules, this glial membrane forms the outer wall of the perivascular space in these regions. In no instance did India ink or colloidal mercury sulfide pass through the meshes of this network. These findings indicate that an interfibrous barrier, impermeable to a finely divided colloidal suspension may exist and lends support to Held's ('09) belief in a neuroglial syncytium.

As the glial membranes of the arterioles and venules are traced along the blood vessels toward the surface of the brain they become increasingly dense. To the sparse networks of fibers surrounding the smaller vessels are added the perivascular astrocyte processes of the neighboring cells. The network fibers change into heavy cords and bands, the meshes become smaller and smaller, and as the larger vessels are approached, the membrane gradually becomes a heavy, many-layered felt-like sheath (figs. 7 and 8). As has been described above, there is no outer mesothelial cell membrane surrounding the distal extent of the arteries. Here, as well as around the arterioles and venules, the glial membrane is the outer limiting membrane of the perivascular space.

Perivascular space proper

Description of the perivascular spaces themselves are based upon the study of colored injection materials which have entered and filled or outlined the spaces. No true spaces except for the entrance to the perivascular space and artefacts can be distinguished in the uninjected specimens. The openings of the perivascular spaces into the subarachnoid space are not the funnel shaped structures usually described but are broad shallow depressions or dimples around the blood vessels.

Surrounding the veins the spaces are at first slightly dilated, and taper quickly from 8 to 10 micra to 2 to 4 micra in width (fig. 6). As they penetrate the brain substance they maintain approximately the same dimension. Where the veins receive tributaries there are often widenings of the spaces. Instead of conforming to the surface of the vessel unions the outer walls of the spaces exhibit concavities or convexities (fig. 10). Around the venules (fig. 8) the spaces narrow to 1 micron or less and beyond this point no carbon particles are seen. The colloidal mercury sulfide, however, penetrates beyond this point.

The perivascular spaces around the arteries entering the brain substance (fig. 5) are not dilated as they are around the veins. Here they are about 4 micra in diameter and continue to narrow as the arterioles are approached. At the arteriole the spaces, as around the venules, are only ½ to 1 micra in width.

Trabeculae extending across the perivascular space between its two walls were not seen. Lined perivascular spaces do not exist about capillaries. The lined spaces surrounding arterioles and venules terminate at the capillaries and open into the interstitial tissue fluid spaces of the brain itself. The colloidal mercury sulfide particles are seen, however, surrounding the capillaries (fig. 11). Dehydration of the brain by increased osmotic pressure of the blood has caused the loss of tissue fluid, and its flow has carried the colloidal particles to the surface of capillaries.

Artefacts

The most often seen artefact is the space between the sheath of glial fibers and the brain substance (figs. 6, 8, 10, 11, 18 and 19). This was first described by Held ('19). Its width may vary from a few micra to double the diameter of the vessel it surrounds. This space extends centrally from the pia on the brain's surface to and including the capillaries. Surrounding the terminations of arterioles and venules this artefact space of Held may be continuous with the termination of the true perivascular space.

The often described pericellular or perineuronal space is believed to be a shrinkage artefact surrounding the nerve cells and often their processes. It is easily demonstrated as an extension of the shrinkage space of Held (fig. 10). It is about those nerve cells whose bodies or processes are in contact with any part of the space of Held. Since all nerve cells are in close association with blood vessels and capillaries, this pericellular artefact may surround any nerve cell in the entire central nervous system.

Observations disclose two other incomplete and only occasionally seen spaces. One may be called an adventitial space (fig. 19), however, it appeared to be between the adventitia and the inner mesothelial cell lining of the true perivascular space. The other potential shrinkage space (fig. 19) is between the outer lining wall of the true perivascular space and the glial membrane. This artefact is erroneously called the space of His. Repetitions of the His multiple puncture technique did not inject this space, but did result in the injection of the "space of Held" and the pericellular spaces (figs. 12, 13 and 14).

DISCUSSION

In order to discuss properly the problem of perivascular spaces, it becomes necessary to attempt to clarify the confusion existing in some of the literature. This misunderstanding has arisen principally from lack of precise anatomical localization and definition of terms.

Since the adventitia of the blood vessels in the brain is thin, and since the extensions of the pia and arachnoid around those vessels are difficult to distinguish, it is easily seen why Virchow and Robin described intra-adventitial rather than extra-adventitial spaces. Their descriptions are undoubtedly of the perivascular spaces and not of adventitial artefacts. Key and Retzius discovered the pia and arachnoid extensions about the blood vessels and described them as lining a space. However, they designated that space as intra-adventitial as did Virchow and Robin. Whether this space is intra- or extra-adventitial depends upon a definition of the limits of the adventitia.

His described spaces which he himself thought at first to be artefacts. The spaces he illustrated and described are identical with those ordinarily seen and identified as artefacts in dehydrated histological or pathological preparations. Repetitions of the His technique, commonly used for the injection of lymphatics and with which the author became familiar in the injection of lymphatics of the heart (Patek, '38) were repeated. The results indicate definitely that His injected a potential space or artefact located between the glial membrane and the neural tissue and not between the outer wall of the perivascular space and glial membrane as was thought by Key and Retzius, and which has subsequently been called the ''space of His.'' The space His actually injected was first recognized and described as an artefact by Held in 1909 and is known in the literature as the ''space of Held.''

The method of Weed, and McKibben and Weed, who used the increased osmotic pressure of the blood to fill perivascular spaces with a true solution of potassium ferrocyanide and iron-ammonium citrate which was precipitated as Prussian blue, has already been described. These workers succeeded in injecting perivascular, pericellular and perifibrillar spaces. Although the true solution of potassium ferrocyanide and iron-ammonium citrate injected was isotonic with cerebrospinal fluid under normal conditions, the altered osmotic pressure of the blood undoubtedly altered the relative isotonicity of the injected substances and they diffused through the perivascular space membranes into the artefact space of Held and thence along paths of least resistance around nerve cells and fibers. Weed himself was unable to repeat his findings when India ink was substituted for the true solution. Repetition of the Weed technique by the author resulted in Prussian blue granules being precipitated within the nerve cells and in one instance the granules were precipitated within nerve cell nuclei.

The present study of the perivascular spaces of the brain indicates that there are true extra-adventitial perivascular spaces. These spaces are the same as those described by Virchow, Robin, and by Key and Retzius; though they thought them to be intra-adventitial. The walls of these spaces terminate at the beginning of the capillaries and their fluid contents become continuous with the tissue fluid of the brain. It may also be concluded, therefore, that no anatomical barrier exists between brain capillary wall and neural tissue and that through the perivascular spaces the neural tissue fluid is directly continuous with cerebrospinal fluid.

CONCLUSIONS

1. The true perivascular spaces extend from the subarachnoid space to the beginning of the capillaries where their contents become continous with the neural tissue fluid.

2. The arachnoid gives rise to mesothelial cell membranes which form the inner lining walls of the perivascular spaces. They extend to the capillary terminations of the arterioles and venules.

3. The pia gives rise to mesothelial cell membranes which form part of the outer lining walls of the perivascular spaces. Around arteries these cellular membranes extend for only a very short distance. Around veins they extend to the terminal parts of the venules. The terminations are irregular and appear to form fenestrated membranes.

4. The glial membranes begin at the central ends of the arterioles and venules and continue to the brain surface. They form the outer walls of those parts of the perivascular spaces which possess no outer mesothelial lining.

5. Four potential, shrinkage or pathological, artefact spaces may be identified. The first is between the blood vessel adventitia and the inner wall of the perivascular spaces. The second, erroneously called the "space of His," is between the outer mesothelial cell walls of the perivascular spaces and the glial membranes. The third is the "space of Held" which lies between the glial membranes and the brain substance. The fourth is the pericellular space which is continuous with the artefact "space of Held."

6. The capillaries of the brain, except for the attachment of astrocyte feet, are anatomically similar to the capillaries of other tissues. No anatomical barrier exists between capillaries of the brain and brain tissue.

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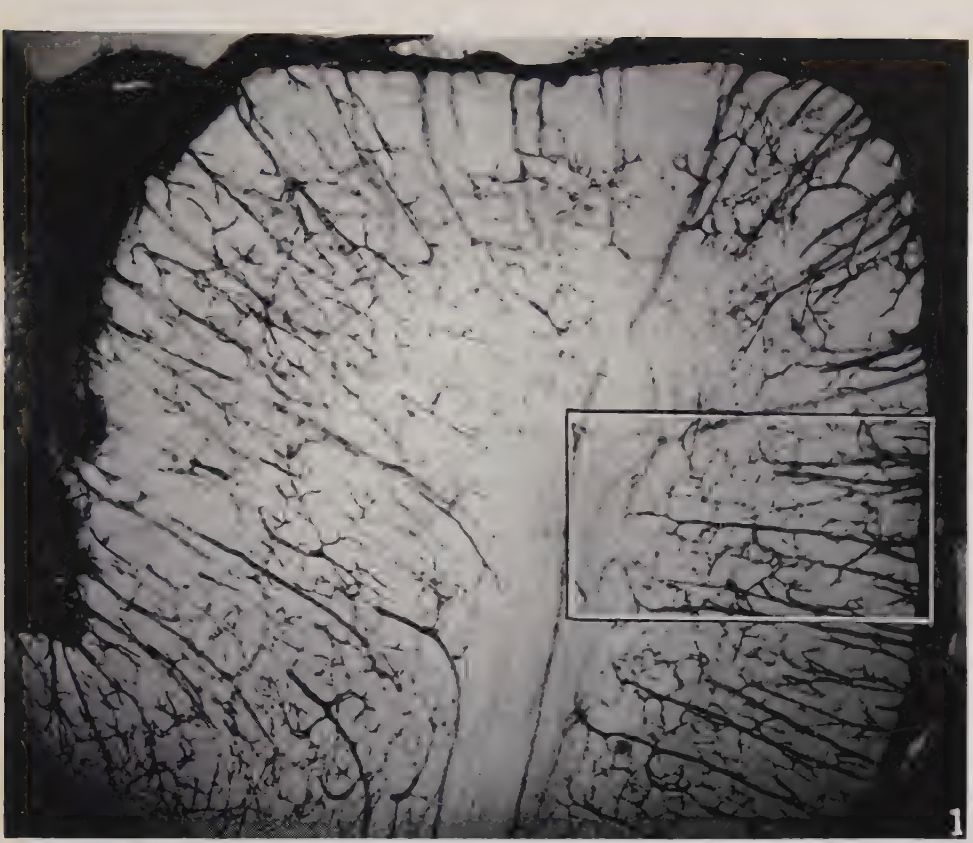
PLATE 1

EXPLANATION OF FIGURES

1 Section through a gyrus of the cerebral cortex showing the perivascular spaces injected with colloidal mercury sulphide. Unstained, dog, × 32.

2 Enlargement of area outlined in 1. Unstained, dog, × 100.



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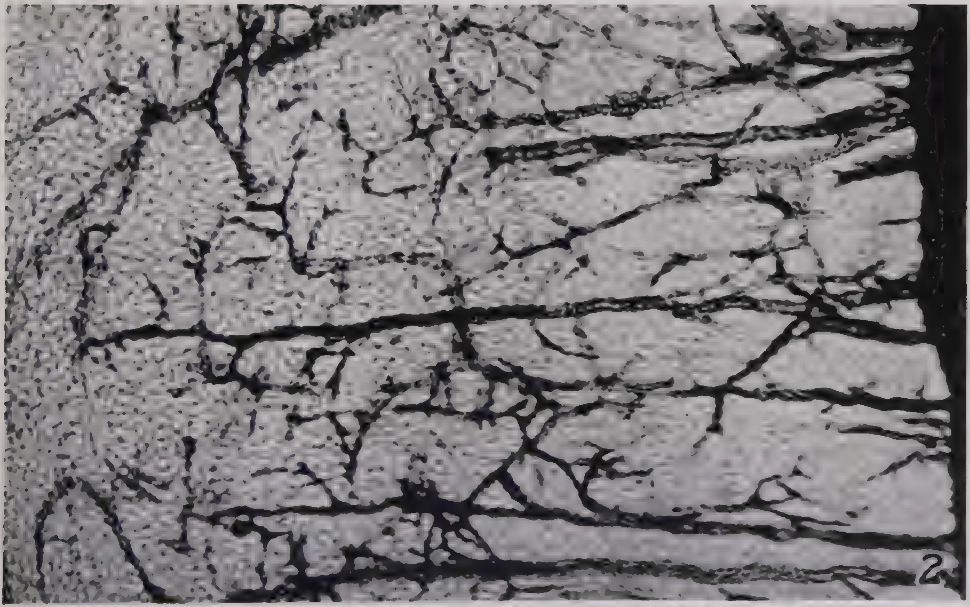

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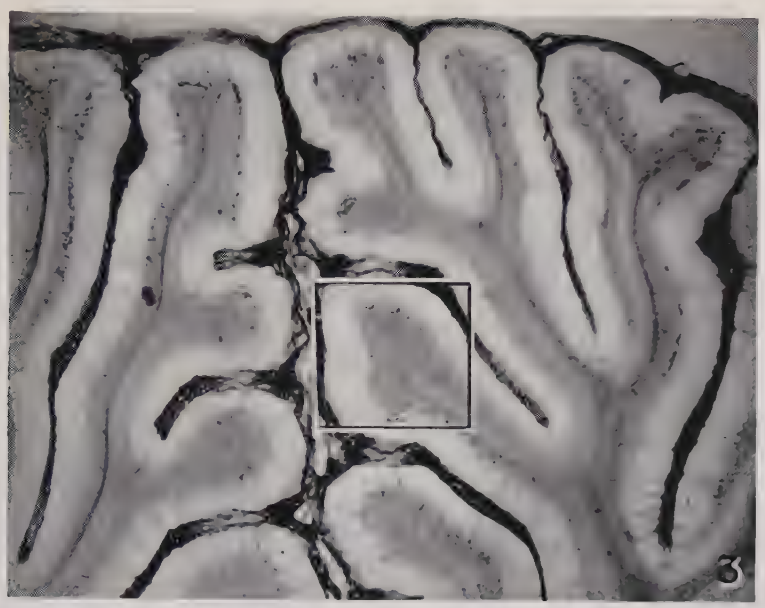
PLATE 2

EXPLANATION OF FIGURES

3 Section through cerebellar gyri showing the perivascular spaces injected with colloidal mercury sulphide. Unstained, dog, × 16.

4 Enlargement of gyrus outlined in 3. Unstained, dog, × 100.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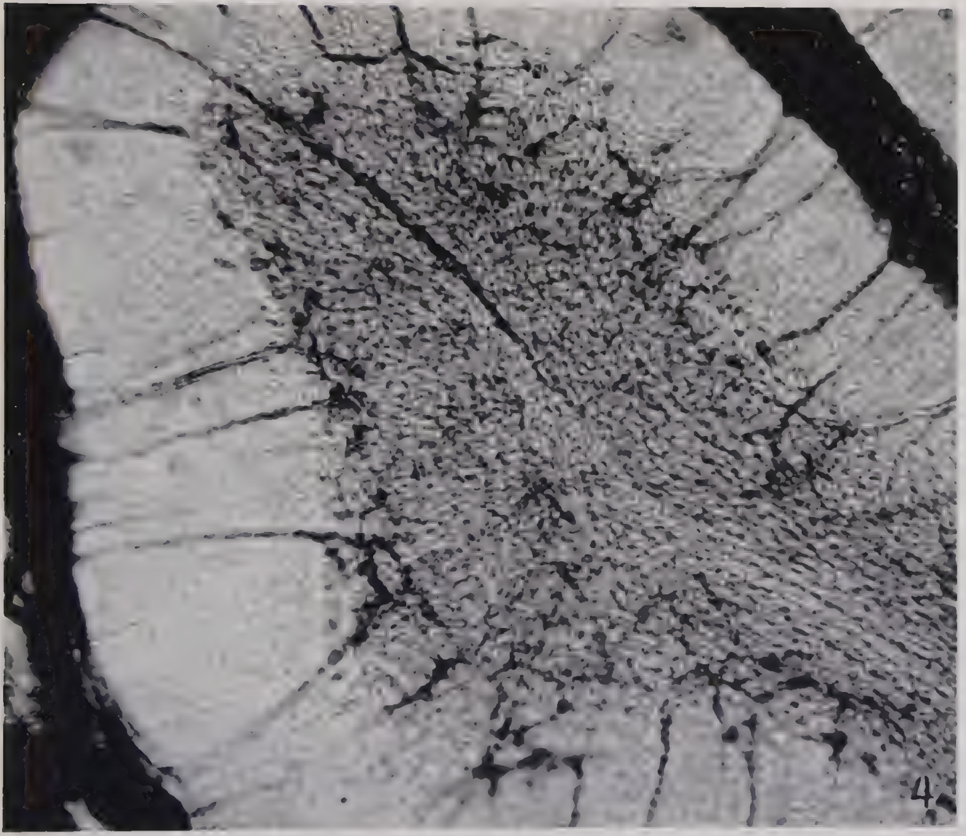
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PLATE 3

EXPLANATION OF FIGURES

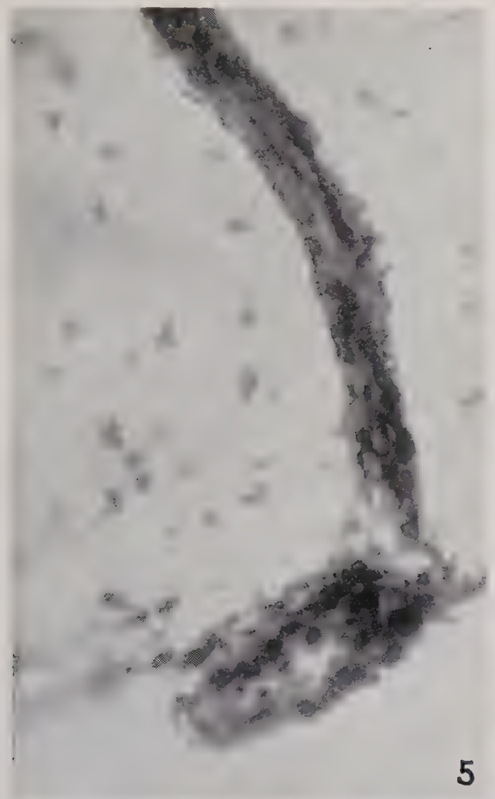
5 Perivascular space filled with colloidal mercury sulphide surrounding an artery entering the cerebral cortex. Gallocyanin, dog, × 400.

6 Perivascular space filled with colloidal mercury sulphide surrounding a vein entering the cerebral cortex. Clear area is artefact space of Held. Gallocyanin, dog, × 400.

7 Colloidal mercury sulphide filled perivascular space about an arteriole within the cerebral cortex. The sharp line on the outer surface of the vessel is the injected material. Note the lack of nuclei external to this line. Gallocyanin, cat, × 450.

8 India ink filled perivascular space about a venule within the cerebral cortex. The clear space peripherally is the artefact space of Held. Gallocyanin, rabbit, × 650.

9 Macrophages in the mesothelial cell membrane forming the outer wall of the perivascular space about this cerebral vessel have phagocytozed colloidal mercury sulphide; the clear centers of the macrophages are the unstained nuclei. Unstained, cat, × 170.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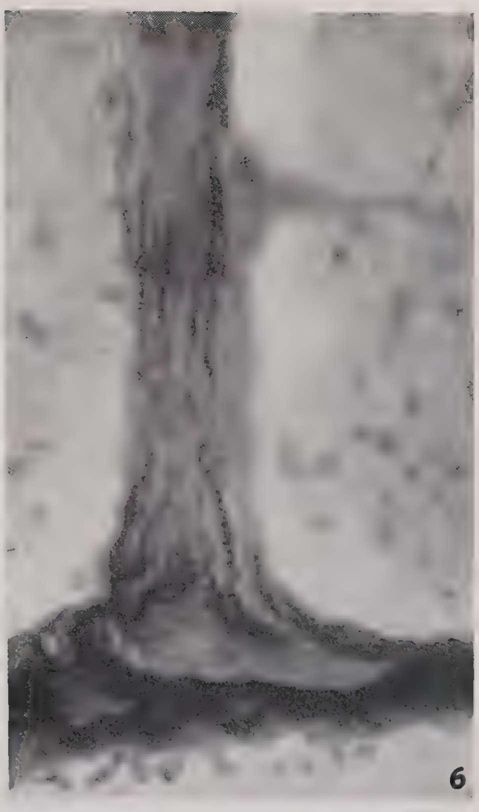
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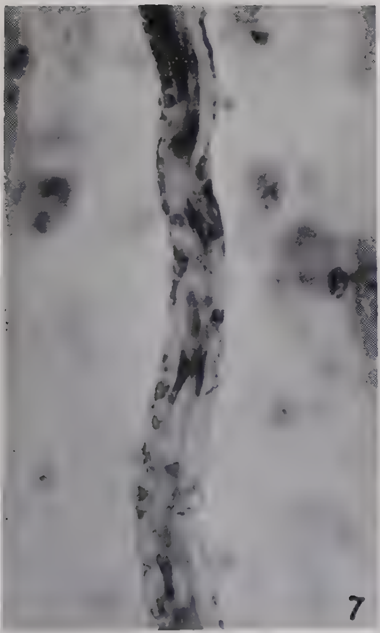
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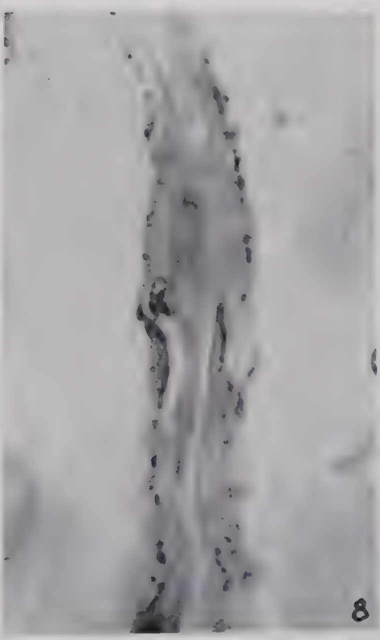
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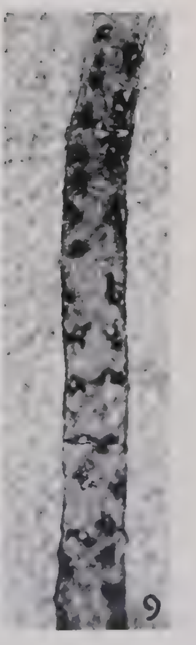
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PLATE 4

EXPLANATION OF FIGURES

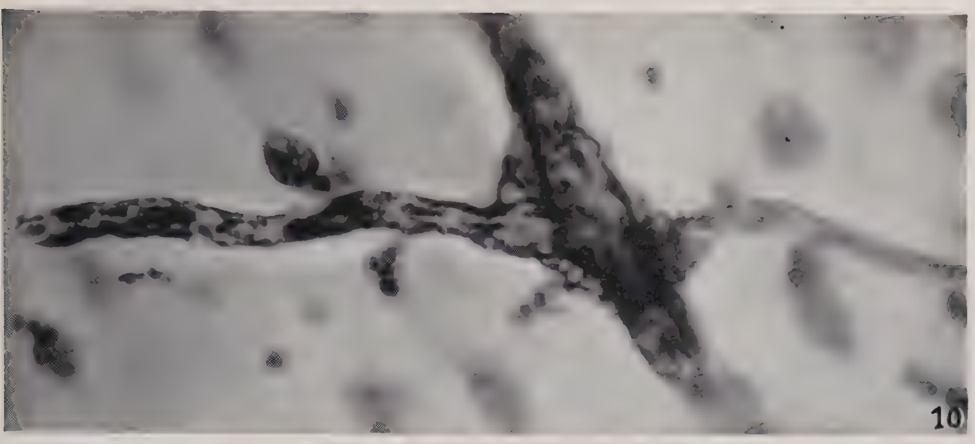
10 The dilations of the perivascular space at the junction of two venules with a small vein have been outlined by the injected colloidal mercury sulphide. Note the clear artefact space of Held external to the perivascular space of the left venule and its continuity with a pericellular space. Gallocyanin, dog, × 550.

11 Colloidal mercury sulphide adherent to the outer surface of this capillary in the cerebral cortex outlines it and has also been phagocytozed by two pericapillary histiocytes. The clear space on either side of the capillary is an extension of the artefact space of Held. Gallocyanin, dog, × 900.

12 By the multiple puncture method of His, India ink was injected into the artefact space of Held about this venule. Gallocyanin, rabbit, × 700.

13 As in figure 12, India ink is in the artefact space of Held and also in the adjoining artefact pericellular space about a nerve cell and its satellite oligodendrocyte. Gallocyanin, rabbits, × 1200.

14 As in figure 13, again India ink is in the single artefact pericellular space about two nerve cells. Gallocyanin, rabbit, × 1200.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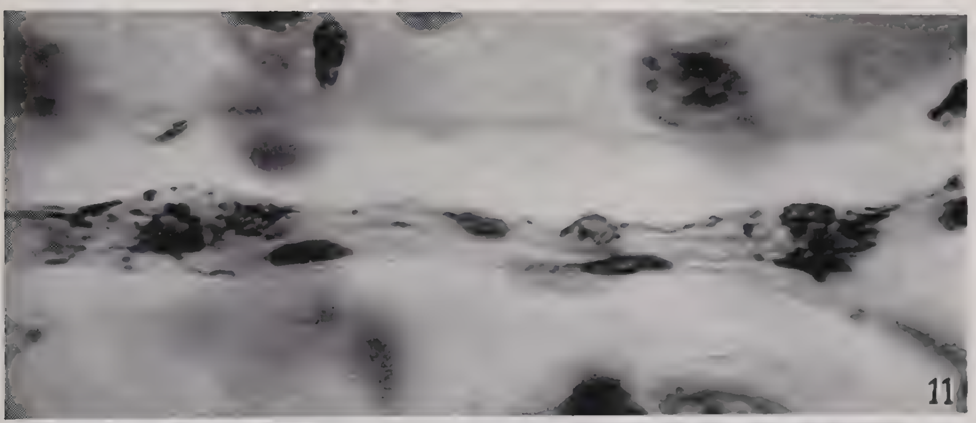
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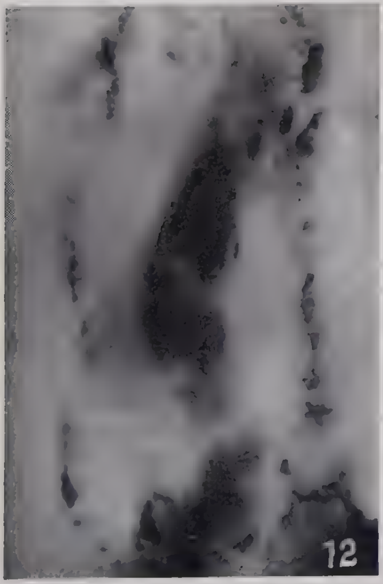
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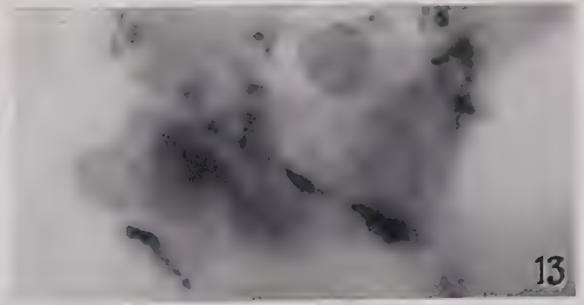
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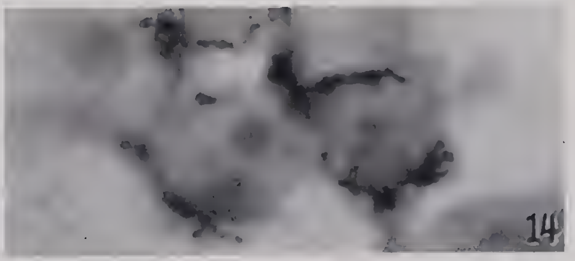
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PLATE 5

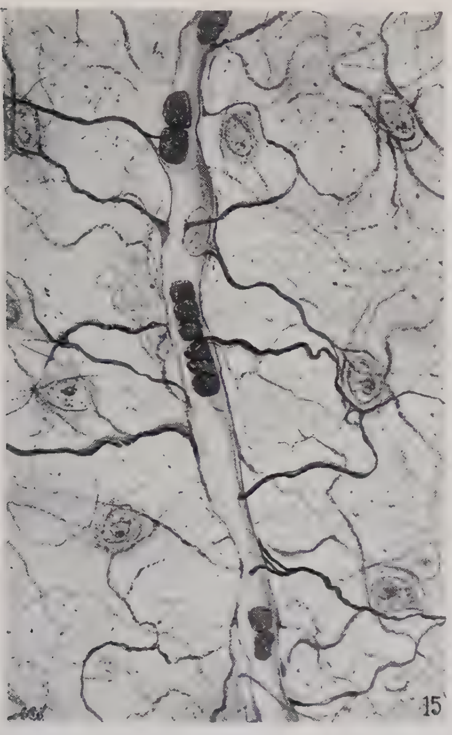
EXPLANATION OF FIGURES

15 Camera lucida drawing of a capillary and attaching perivascular feet of fibrous astrocytes. Rio-Hortega's silver carbonate (lithium), cat, × 110 .

16 Camera lucida drawing of a loose meshed glial membrane surrounding the perivascular space of an arteriole. Rio-Hortega's silver carbonate (lithium), cat, × 750.

17 Camera lucida drawing of a dense fibrous glial membrane around a large artery. Note artefact space of Held and glial fibers passing through it. Note also the glial fibers torn by the shrinkage which produced the artefact. Rio-Hortega's silver carbonate (lithium), cat, × 750.

18 Camera lucida drawing of a cross section of a dense fibrous glial membrane about an artery similar to that llustrated in figure 17. Rio-Hortega's silver carbonate (lithium), cat, × 750.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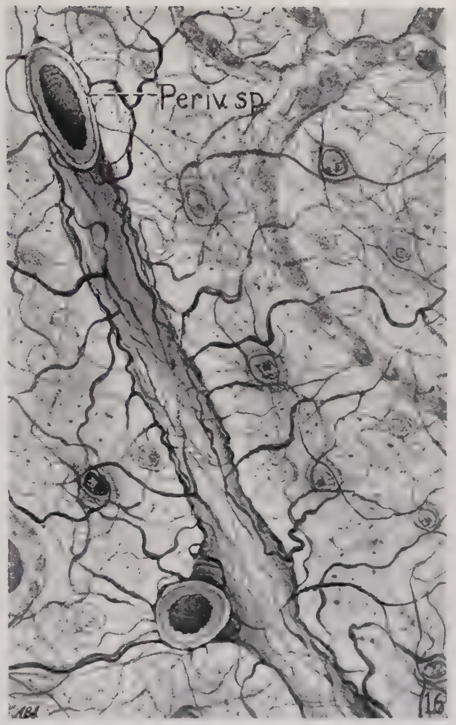

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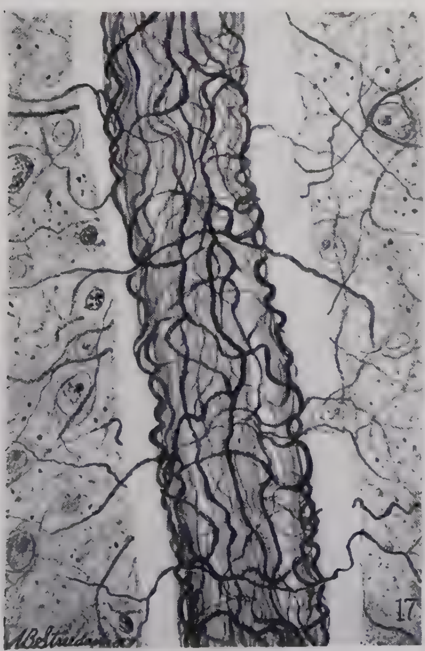
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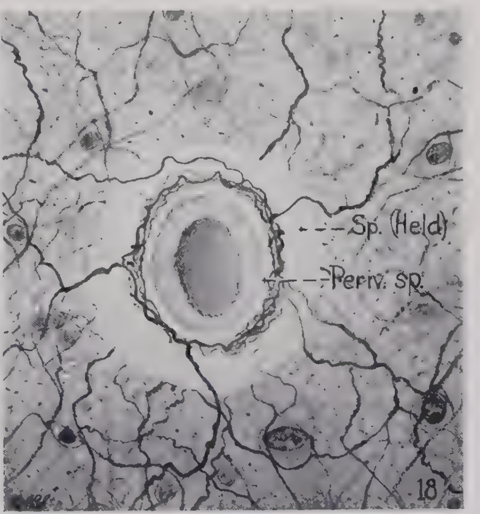
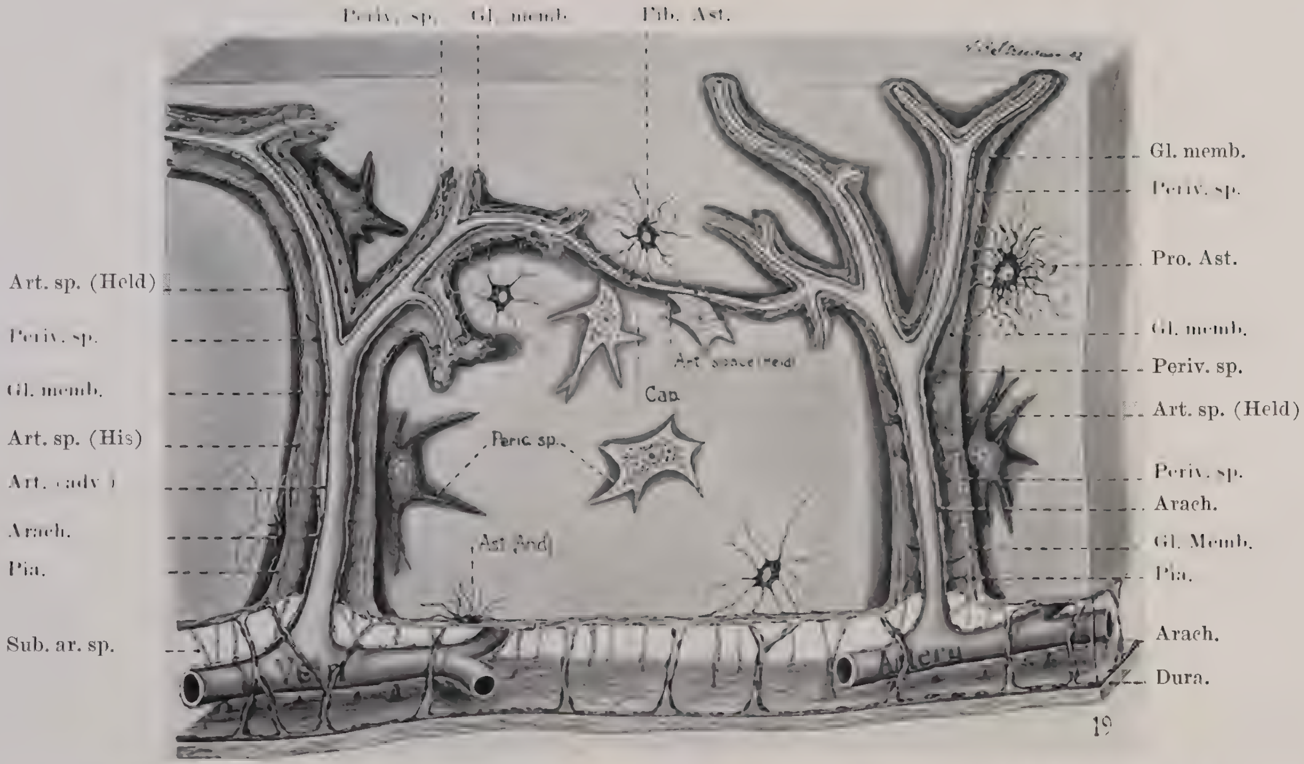

Sp. (Held)
Periv. sp.
18


Periv. sp.
Gl. memb.
Fib. Ast.
Art. sp. (Held)
Periv. sp.
Gl. memb.
Art. sp. (His)
Art. (adv.)
Arach.
Pia.
Sub. ar. sp.
Gl. memb.
Periv. sp.
Pro. Ast.
Gl. memb.
Periv. sp.
Art. sp. (Held)
Periv. sp.
Arach.
Gl. Memb.
Pia.
Arach.
Dura.
Art. space (Held)
Cap.
Peric. sp.
Ast. (And.)
Vein
Artery
19

19 Diagrammatic drawing of the extent of the perivascular spaces, their coverings, associated cells, and associated shrinkage or pathological art facts: Arach., arachnoid; Art., (Adv.), adventitial artefact; Art. sp. (Held), artefact space of Held; Art. sp. (His), artefact space of His; Ast. (And.), astrocyte of Andriezen; Cap., capillary; Fib. Ast., fibrous astrocyte; Gl. Memb., glial membrane; Peric. sp., pericellular space; Periv. sp., perivascular space; Pro. Ast., protoplasmic astrocyte; Sub. ar. sp., subarachnoid space.

THE EFFECTS OF RETROGRADE DEGENERATION UPON REFLEX ACTIVITY OF VENTRAL HORN NEURONS [1]

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FOUR TEXT FIGURES AND ONE PLATE (THREE FIGURES)

INTRODUCTION

Certain aspects of the problem of the effect of neurotropic viruses on cellular activity indicate that retrograde degeneration (or the so-called axonal reaction) may offer a clue to the nature of the effects of such agents. The most important observation is that made by Howe and Bodian ('42) in discovering that cells previously degenerated by section of peripheral nerves are no longer subject to invasion by anterior poliomyelitis virus. The observation of Olitsky ('39) in his study of avian encephalomyelitis that, in addition to the classical necrotic changes induced in neurons by this virus, certain cells respond by a milder reaction closely resembling the chromatolytic picture of retrograde degeneration seems also to indicate that the chromatolysed cell has certain metabolic processes in common with the diseased cell. It is noteworthy that Olitsky's observation was corroborated by Sabin and Ward ('41) in a study of anterior poliomyelitis. On the basis of these fragmentary signs a study has been made of the functions of cells caused to degenerate by section of their axons.

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[1] Aided by a grant from the National Foundation for Infantile Paralysis.

[2] Present address: Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minnesota.

That there is a change in function of nerve cells following severance of their peripheral processes is shown by the investigation of Acheson, Lee and Morrison ('42). They reported that following high or low section of the phrenic nerve in cats a decrease in the respiratory discharges occurs. This shows a time course paralleling that usual in retrograde degeneration with recovery in about 2 months. The phrenic nerve neurons involved, however, showed little signs of chromatolysis.

A preliminary note (Campbell, '43) has indicated the method used in this study, namely the employment of the segmental spinal reflex as a test of the activity of the involved neurons. It was further pointed out that the proprioceptive component of this reflex picture — two neuron reflex of Renshaw ('40) — was the part affected by the degeneration. The findings which relate the phenomenon of the suppressed proprioceptive reflex to the condition of the anterior horn cell were not presented. As the data is now available, a more complete report will be made.

MATERIALS AND METHODS

Cats and rhesus monkeys were used in these experiments. Under nembutal anaesthesia several different types of operations were attempted. In some the tibial and peroneal nerves were sectioned in the popliteal fossa. In others the whole sciatic complex was cut at the greater sciatic foramen. Another series was prepared with root sections, dorsal, ventral, or both, of one or more segments. All operations were performed on the right side and the left side served as simultaneous control for both the anatomical and the physiological observations.

After the period of degeneration had elapsed, the animals were again anaesthetized, this time with Dial, the spinal cords laid bare and covered with mineral oil. Stimulation of the peripheral nerves or of the dorsal roots was effected by means of a condenser discharge which was sweep-synchronized through an electronic relay. In the later experiments of this series, three such channels were available. Recordings were made photographically of the ventral root action potentials after they were amplified (with a condenser coupled amplifier of long time constant) and fed into a cathode ray oscillograph.

Following the recordings, the animals were dispatched by bleeding and the tissues fixed either by perfusion with neutral formalin or by immediate immersion in that fluid. For staining, cresyl violet and the Bodian silver technic were used.

RESULTS

The normal potential change near the killed end of a ventral root following stimulation of the corresponding dorsal root is shown in figure 1. This has been lucidly discussed by Lloyd ('43) who demonstrated that the first mode of the curve, the high spike which shows a very short central delay, is due to stimulation of fibers transmitting muscle sensibility, while the delayed and diffuse second mode is the reaction to stimulation of the fibers of cutaneous sensibility or of pain. The proprioceptive reflex is returned to the muscle field in which the stimulus arose; the cutaneous reflex, however, is not confined to any narrow field and is observable even in the segments bordering on that stimulated.[3]

Figures la and b illustrate the effect of retrograde degeneration upon the potentials elicited from the ventral roots by peripheral stimulation. This animal (cat no. 75) had had the right tibial and peroneal nerves sectioned in the popliteal fossa 34 days prior to the recording. In this experiment, the right L7 and Sl ventral roots were cut intradurally and the potentials elicited by stimulation of the tibial and peroneal nerves were recorded from the central ends. Thus, antidromic stimulation of the motor cells of the segments studied was

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[3] With variations in temperature, depth of anaesthesia, and of the general condition of the animal, the size of the two components varies somewhat. In the records upon which this account is based, the necessity of employing means of exactly duplicating conditions in each suceeding experiment was dispensed with by the expedient of using as simultaneous control, the unoperated side of each animal. Nevertheless, care was taken to keep the conditions surrounding the experiments within usual physiological practice.

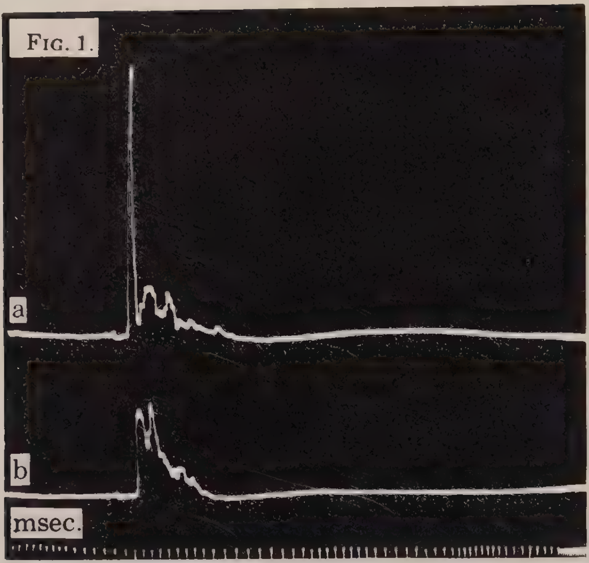

Fig. 1 Potentials recorded from the central ends of the cut seventh lumbar ventral roots in response to stimulation of the combined tibial and peroneal nerves. a. Normal side. b. From the side in which the tibial and peroneal nerves had been sectioned more distally 34 days earlier. Time in milliseconds.

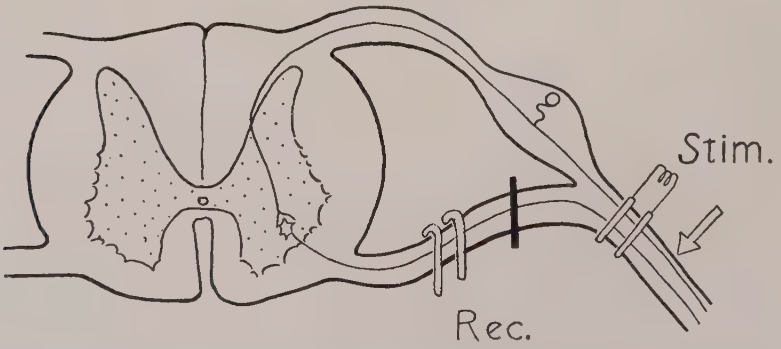

Fig. 1. c. Diagram of experimental procedures. Arrow marks site of nerve section. Points of stimulation and of recording indicated. Heavy line denotes section of ventral root at the time of the recording.

avoided, and the reflexes recorded represent those contingent upon impulses entering the cord via the dorsal roots. It will be seen (fig. 1a) that after 1.5 misec. occupied by peripheral and central conduction of the activity, the unaffected side of the cat shows a high peak representing the proprioceptive reflex, followed by later and more diffuse activity which may be referred to as the cutaneous response.

The degenerated right side of the preparation gave a series of potentials, figure 1b. Here it is seen that the early proprioceptive peak is entirely lacking from the reflex picture, though the later flexor response is present. (It is to be observed in this instance as well as in most others illustrated that where the proprioceptive response is deficient because of retrograde degeneration, the flexor response is larger and of somewhat different contour than in the controls. This can not be given any significance for in all probability it merely shows the effects of the absence of the subnormality of the motor neurons usually existing in the time immediately following the proprioceptive spike. Thus it may be seen in the undegenerated preparation, that repetitive stimuli, by decreasing the size of the proprioceptive spike, bring about a heightening of the cutaneous component of the response through a similar release).

Figure 2 a and b shows the appearance of the first sacral ventral root potential following stimulation of the corresponding dorsal root rather than of the peripheral nerve. These records, from monkey no. 6, were made 41 days after the entire sciatic complex had been sectioned on the right side. The normal control, 2a, shows the large proprioceptive spike appearing 2.5 msec., from the shock artefact, its decline in 1 msec, after which a long drawn out cutaneous response is visible. On the degenerated side of this animal (fig. 2b) the loss in the early component is conspicuous. The small deflection indicates the reflex activity of the muscle fields supplied by this segment which were untouched by the operation, i. e., intrinsic back and hip muscles. Much more conspicuous is the cutaneous reflex which in this instance is as large as the proprioceptive reflex on the normal side.

From the experiments outlined above, it is not apparent where the pathological process which results in this deficiency is to be found. Following section of a peripheral nerve, degenerative changes are seen both in the dorsal root ganglia and

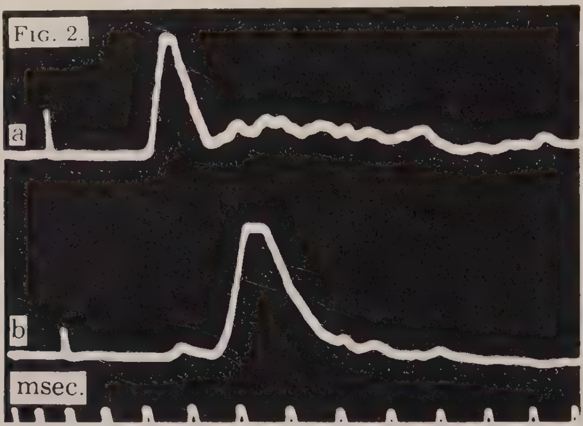

Fig. 2 Potentials recorded from cut ends of first sacral ventral roots of rhesus monkey in response to stimulation of corresponding dorsal roots. a. Normal side. b. From the side in which the whole sciatic complex had been sectioned 41 days previously. Time in milliseconds.

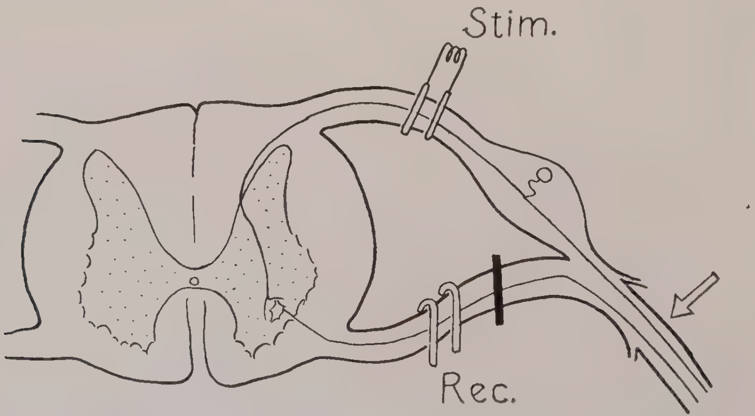

Fig. 2 c. Diagram of experimental procedures. Arrow marks site of nerve section. Points of stimulation and of recording indicated. Heavy line denotes section of ventral root at time of recording.

in the primary motor neurons. On the basis of Renshaw's convincing demonstration that this early reflex activity does not involve interneurons (loc. cit.) we may safely disregard the possibility of transneuronal degeneration in the intermediate nuclei of the spinal cord. To examine the matter further, a number of experiments were performed in which roots instead of nerves were sectioned. No information was gained from section of the dorsal root alone. The central processes of the root degenerated and became nonconductile so that no segmental reflex could be elicted.

The experiments in which the ventral roots were sectioned show that the sensory system is not the site of this phenomenon. Illustrated in figure 3 are the results of such an experiment. On the side of the root lesion, the proprioceptive reflex is seen to drop out entirely. It is plain, therefore, that it is the change within the motorneuron which is brought about by the section of its process that is immediately correlated with the reflex deficiency.

Additional indication that changes in the sensory system are not responsible for the phenomenon may be found in the experiments in which conduction through the fibers of the affected dorsal root ganglion cells was tested. Figure 4 shows the spike recorded in the dorsal root in response to a single stimulus applied to the tibial and peroneal nerves. The same nerves had been sectioned on the right side 12 days previously. It must be supposed that the spike in figure 4b is the product of activity of fibers all of which had degenerated cell bodies. Thus the fact that conduction occurs in these cells is established. The slight variations in the latency of the two spikes and of their size is not considered of significance.

The loss of proprioceptive reflexes following retrograde degeneration is limited to pathways involving the motor neurons affected. Thus, in the cat, when the tibial and peroneal nerves were cut the nuclei for the hamstring muscles were not affected and responded to stimulation of the muscle sensory fibers from these muscles with their normal pattern.

In many instances, however, there are indications of some process whereby the nuclei and segments adjoining the degenerated fields show alterations in their response. This is particularly noticeable in the monkey and work now in progress on this point will be discussed in a later paper.

FIG. 3.

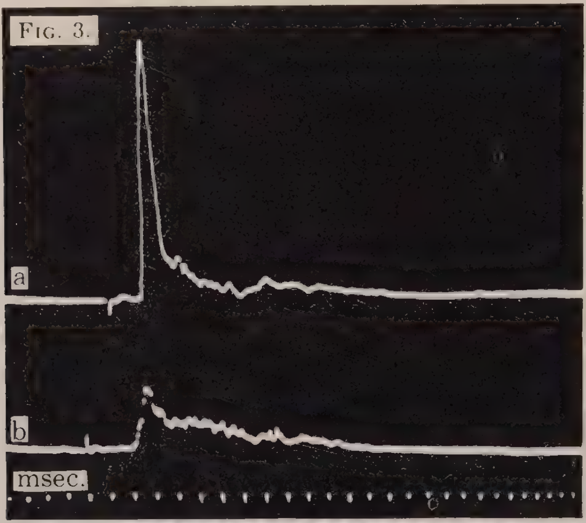

Fig. 3 Potentials recorded from the cut ends of the seventh lumbar ventral roots of a cat in response to stimulation of the corresponding dorsal root. a. Normal side. b. From side in which the ventral root had been sectioned more distally 10 days earlier. Time in milliseconds.

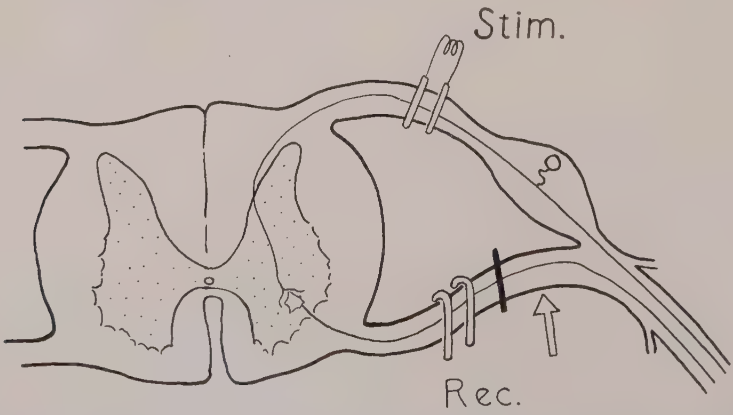

Fig. 3 c. Diagram of experimental procedure. Arrow marks site of ventral root section. Points of stimulation and of recording indicated. Heavy line denotes further sectioning of ventral roots at time of recording.

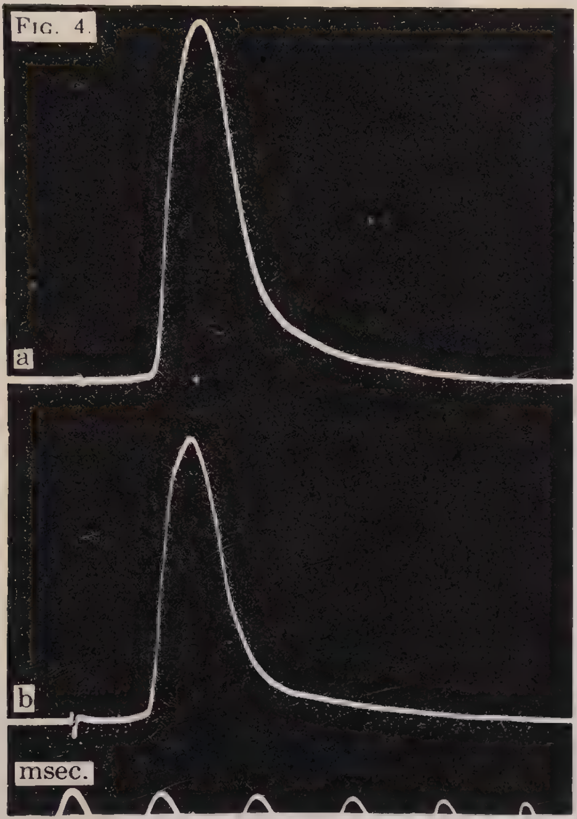

Fig. 4 Potentials recorded from distal ends of cut seventh lumbar dorsal roots of a cat in response to stimulation of the combined tibial and peroneal nerves. a. Normal side. b. From side in which which the tibial and peroneal nerves had been sectioned more distally 12 days earlier. Time in milliseconds.

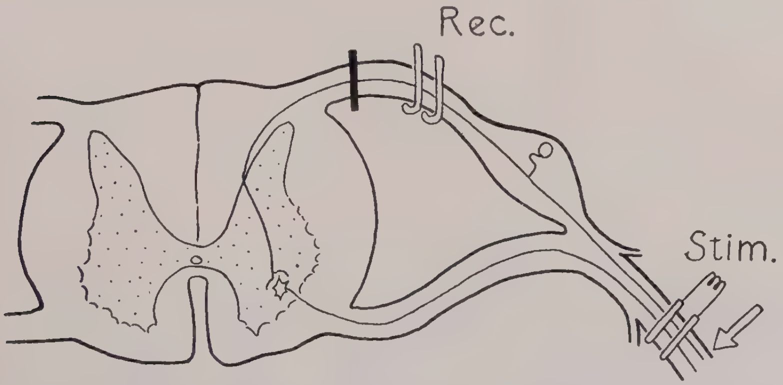

Fig. 4 c. Diagram of experimental procedures. Arrow marks site of nerve section. Points of stimulation and of recording indicated. Heavy line denotes section of dorsal root at time of recording.

DISCUSSION

From the work outlined above, one may conclude that the physiological state of the chromatolysed motor cell is such that it can still react to incoming stimuli though unable to respond in normal fashion to the proprioceptive component. That a difference of this sort should exist is rather surprising and may be taken as an indication that some difference, either quantitative or qualitative, between the excitatory processes set up at the locus of the motorneuron must occur. In view of the speculative nature of our ideas on the mechanism of transneuronal excitation it seems impossible to deduce anything helpful from the facts as now known. It is important, however, to remember that the proprioceptive reflex is not dependent upon the firing of the intermediate spinal nuclei and, not being reinforced by intraspinal mechanisms, probably represents the response of the motor nuclei to an extremely powerful excitatory process. This can be assumed to rise within a very short period of time, possibly 1 millisecond. The cutaneous reflex, on the other hand, is the response of the motor cells to some altered state set up, arising more slowly because of the nuclear delay (Lloyd, '41, p. 538, footnote) at the intermediate regions of the gray matter. It is conceivable, then, that the altered cell may have lost its ability to respond to excitation of short duration while quite able to respond to those arising more slowly and lasting longer.

SUMMARY

1. Section of peripheral nerves or of ventral roots in cats and monkeys leads to the loss of the proprioceptive component of the segmental spinal reflex during the period of chromatolysis.
2. This is directly correlated with the alteration of the ventral horn cell and does not stem from degeneration in the sensory system.
3. Conduction occurs from the peripheral to central fibers of the dorsal root ganglion cells after retrograde degeneration induced by nerve section.

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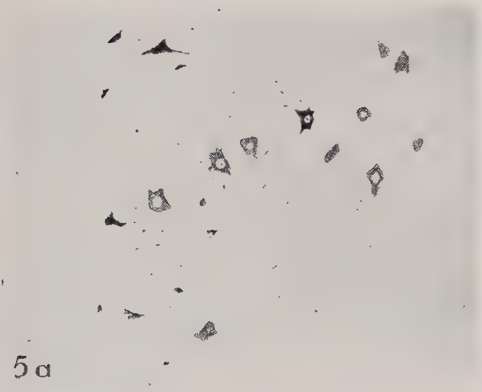
PLATE 1

EXPLANATION OF FIGURES

5 Cells of the ventral horns of the sixth lumbar segment of cat, no. 80 (cf. fig. 3). The right sixth lumbar ventral root had been cut 30 days previously. a. Normal side. b. Degenerated side. In addition to chromatolysis. some of the affected cells show eccentric nuclei. Cresyl violet.

6 Cells of the ventral horns of the seventh lumbar segment of cat no. 75 (cf. fig. 1). The right tibial and peroneal nerves had been cut 34 days previously. a. Normal side. b. Degenerated side. The affected cells are characterized by paler staining and dustlike granules. Cresyl violet.

7 Cells of the ventral horns of the first sacral segment of monkey, no. 6 (cf. fig. 2). The entire sciatic complex had been cut on the right side 41 days previously. a. Normal side. b. Degenerated side. The eccentric nuclei are more frequently shown in the monkey than in the cat.



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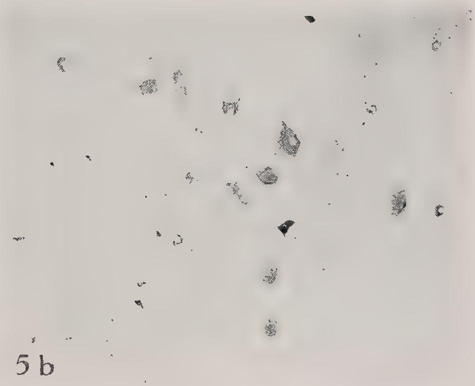

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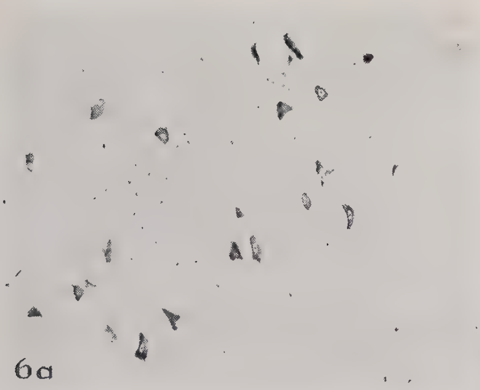

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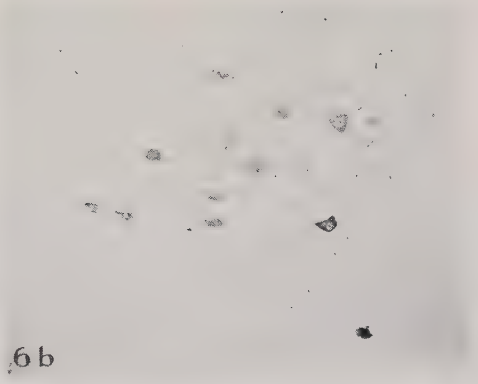

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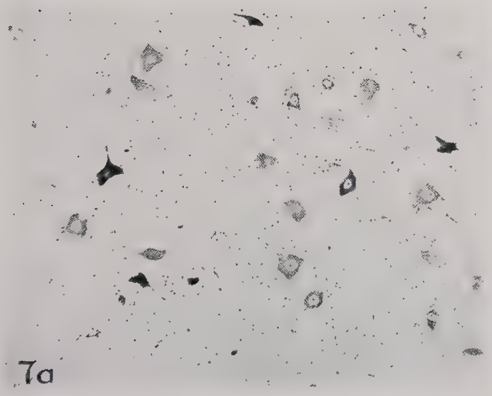

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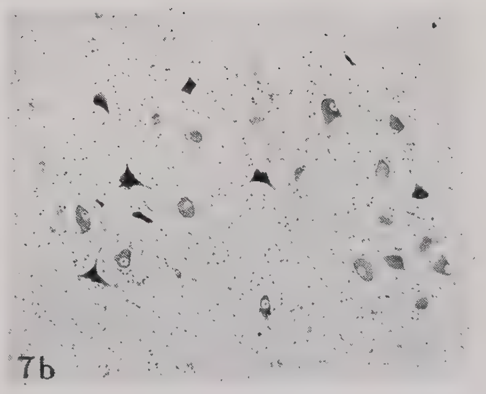

7b

A CYTOLOGICAL STUDY OF THE DIURNAL CYCLE OF THE LIVER OF THE MOUSE IN RELATION TO STORAGE AND SECRETION[1]

HELEN WENDLER DEANE[2] [3]
Arnold Biological Laboratory, Brown University, Providence, Rhode Island

THREE TEXT FIGURES AND TWO PLATES (TWELVE FIGURES)

The correlation between changes in the cell organelles and the processes of storage and secretion is one of the fundamental problems of cytology. In the liver many of the activities of the cell are known through biochemical studies, and in certain instances the products of these activities—e.g. bile acids, glycogen, and fat—can be demonstrated histologically. Several suggestions have appeared in the literature of interrelationships between the organelles and the processes associated with alimentation, but no comprehensive study has been undertaken. The present investigation is such a study of the relationship between changes in the mitochondria and the Golgi substance on the one hand and the secretion of bile acids and the storage of glycogen and fat on the other.

Recently Forsgren ('35) and his associates have described reciprocal diurnal cycles in the deposition of glycogen and in the secretion of the bile components (actually the bile acids, because of the method of demonstration). According to their observations the greatest amount of glycogen is present in the liver during the night, whereas the largest amount of bile acid material is secreted during the afternoon. Except for minor shifts in time this cycle is characteristic for the several rodents studied—the rabbit, the rat, and the mouse. In addition, the storage of glycogen occurs initially and most intensely at the center of the lobule, and the secretion of bile acids is continuous at the periphery. On the basis of such observations Forsgren has concluded that there is an antagonism between the deposition of glycogen and the secretion of bile acids by the liver cell. As a result, the quantity of the two substances and their distribution in the liver lobule vary inversely.

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Brown University, September, 1943.

[2] Horton-Hallowell Fellow of Wellesley College, 1942–43.

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From a consideration of the studies of the Stockholm group it was believed that, by killing animals at intervals during the 24-hour period, a separation of the processes of storage and secretion might be effected. Any related changes that might occur in the mitochondria and in the Golgi substance could then be better observed. Since Higgins, Berkson and Flock ('32, '33) had shown that the time of storage is probably dependent upon the time of feeding and digestion, two types of experiment were planned—one in which the animals were allowed to feed at will and one in which they were provided with food for only a short period during the day. It was hoped that by the latter method any antagonism between the two processes would be accentuated.

I wish to thank Prof. J. Walter Wilson for his constant encouragement and many suggestions during the course of the investigation.

MATERIAL AND METHODS

White Swiss mice raised in our laboratory were used for these experiments. After weaning they were fed Rockland Mouse Pellets.

For the uncontrolled diurnal-cycle series eight male mice, 78 ± 5 days of age, were used. The animals were killed at 3-hour intervals during the 24-hour period. For the controlled diurnal-cycle series thirty-two mice were used: sixteen males, averaging 85 days of age, and sixteen females, averaging 92.

The animals were trained to eat from 7 to 10 P.M over a training period lasting 4 days. On the day of the experiment, two males and two females were killed at 3-hour intervals after the beginning of feeding.

For the cytological preparations, the animals were first stunned and then decapitated, and small blocks of liver from the large left-central lobe were quickly immersed in the various fixatives employed.

The techniques of fixation and staining used follow:

(1) For a general fixative, Bouin's fluid was used. The sections were cut at 10 micra and stained in Delafield's hematoxylin and eosin. The preparations were studied for a description of the cells, and were also used for cell measurements.

(2) For mitochondrial preparations, the tissue was fixed in 10% neutral formalin and then postchromed; the sections were cut at 3 micra and were stained by the long Heidenhain iron-alum hematoxylin method (Deane, '42a).

(3) For the fixation of the Golgi substance, Nassonov's modification of Champy's fluid was used, with post-osmication as directed by Kolatchev (Bowen, '28). With Merck osmic acid, it was found necessary to osmicate the blocks of liver for 2 weeks in order to get satisfactory impregnation. The sections were cut at 5 micra; no counterstain was used.

(4) For the fixation of glycogen, a formol-alcohol mixture was adopted. The sections were cut at 5 micra and stained according to the Bauer-Feulgen technique (Bensley, '39).

(5) For the demonstration of fat, the tissue was first fixed in 10% neutral formalin, then imbedded in gelatin, sectioned at 15 micra on the freezing microtome, mounted on slides with gelatin, and stained with sudan IV (Deane, '42a).

(6) For the preservation and staining of the bile acids, the Forsgren ('28) method was employed: the tissue was fixed in 3% barium chloride followed by 10% formalin. The

sections were cut at 5 micra and stained in Mallory's triple connective-tissue stain (0.1% acid fuchsin, acidified to pH 3, 3–5 minutes; aniline blue-orange G mixture acidified to pH 3, 20 seconds). Bile acid granules stain differentially with the acid fuchsin. It was found necessary to adopt the above times in preference to those recommended by Forsgren in order to get sufficient contrast between the granules and the rest of the cytoplasm.

Judgments of the quantity of bile acids, of glycogen, and of fat have been made for each animal studied. The symbol (+) indicates that only scattered cells in the lobule contain the substance in question; one or more +'s indicates that an appreciable quantity is present. An attempt has been made to judge the amount of the material in the lobule as a whole, irrespective of whether it is localized in a zone or is distributed evenly in the lobule. These judgments may serve as an approximate measure of the amount of the substance that could be ascertained by biochemical analysis, since Hall and MacKay ('33) found that they were able to make judgments of the quantity of glycogen in histological preparations which paralleled biochemical-analysis results, and Hodge et al. ('41) have shown that the amount of fat demonstrable in histological preparations corresponds to the neutral fat extractable from the liver.

Since both Noël ('23) and Forsgren ('28) had stated that the cells enlarge during storage it was desirable to obtain some idea of changes in cell size during the 24-hour period. The Bouin-Delafield preparations were used. The number of cells containing nuclei within a square field outlined by a Howard micrometer disc at a magnification of 563× was counted by drawing the nuclei on a ruled sheet. Three counts were made for each animal—one at the periphery of the lobule, one in the middle, and one in the center—and the average count for the unit area was determined. The reciprocals of these numbers, which may be considered to be indicative of cell size, were plotted and the points connected to reveal changes in cell size during the diurnal cycle.

OBSERVATIONS[4]

Uncontrolled diurnal cycle. One mouse was killed every 3 hours during the 24-hour period. The weights of the whole gut and of the stomach alone were taken for each of the animals; the resulting curves appear in figure 1. The larger peak in the weight of each occurs from 9 P.M. to 3 A.M., with a smaller one at 9 A.M. These peaks correspond to the times of greatest activity of the animals. During the night, the food material was located in the stomach and upper gut; it then descended during the day, and by 6 P.M. all intestinal contents were limited to the lower gut. The liver was a dull red color during the afternoon and a brighter red during the rest of the day.

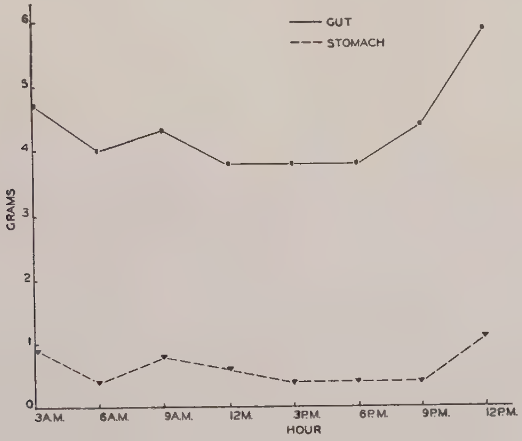

Fig. 1 Uncontrolled-feeding series: change in weight of gut and stomach during the 24-hour period.

The calculations of cell size indicate a maximum at 9 A.M. and a long depression from 12 M. to 3 A.M.

The mitochondria exhibit the zonation within the lobule described in many other studies on the mouse (Noël, '23; Kater, '33; Milletti, '37; Deane, '42a). They are relatively heavy and often short in the peripheral zone (fig. 4), becoming longer and finer toward the central vein (fig. 5). In the few rows of cells adjacent to the central vein the filaments are less numerous and frequently less siderophilic (i.e. less intensely stained with iron-alum hematoxylin) than in the outer part of the lobule.

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[4] Detailed observations are included in the thesis which is available at the John Hay Library, Brown University.

During the 24-hour period the mitochondria of the peripheral zone of the lobule undergo certain changes. At 9 A.M. they are long rods, becoming progressively shorter so that they are virtually spherical at 3 P.M., short rods thereafter. They are never abnormally swollen in more than a few isolated cells. In the middle and central zones of the lobule the chondriosomes show no marked diurnal changes.

From 6 A.M. to 6 P.M. the cytoplasm of the cells, especially in the middle zone, contains irregular siderophilic clumps which are sometimes extensive enough and dark enough to mask the mitochondria (fig. 5). This siderophily reaches its maximum intensity at noon. It is correlated with a marked vacuolation of the cytoplasm. Since neither the siderophily nor the vacuolation of the cytoplasm occur at the surface of the block the condition is apparently an artifact, yet it is so consistent in the series that it seems worthy of notice.

The Golgi substance is composed of strands that may be organized into networks or may appear discrete. During the evening the strands are located primarily in the periphery of the cell; during the rest of the day some pieces are also located near the nucleus. The Golgi material reaches its maximum volume at 3 P.M. It shows a tendency to be more massive in the cells of the peripheral zone of the lobule than in the central and is hardly impregnated at all in the cells near the central vein. From 9 P.M. to 6 A.M. it is only lightly impregnated throughout the lobule.

The quantities of bile acids, glycogen and fat in these livers are given in table 1.

Granules of bile acid are present in all of the cells of the whole lobule throughout the day. Sparse during the morning hours, they become more concentrated until 3 P.M. and remain crowded through the evening. The granules are conspicuously more numerous in the cells of the peripheral zone than in the more central region but are never entirely lacking in the latter.

There is a cyclic variation in the amount of glycogen to be demonstrated in the liver lobule during the day: virtually absent at 9 P.M., it rises steadily in quantity until 9 A.M., then slowly decreases. From 12 P.M. to 3 P.M. it is conspicuously more concentrated in the peripheral part of the lobule, where it is precipitated in the form of large plaques (fig. 10). From 6 to 9 P.M., the few granules of glycogen are most concentrated in the central zone (fig. 12).

TABLE 1

Uncontrolled diurnal cycle: bile acid, glycogen, and fat content of liver in histological preparations.

TIME KILLED	BILE ACIDS	GLYCOGEN	FAT
3 A.M.	++++	+++	+
6 A.M.	++++	++++	++
9 A.M.	+++++	++++++	+
12 M.	++++++	+++++	(+)
3 P.M.	++++++	+++	0
6 P.M.	+++++++	+++	+
9 P.M.	+++++	+	0
12 P.M.	++++++	+++	+++

Fat is present in small quantities from 12 P.M. to 9 A.M. and may be either limited to the central zone or more concentrated in this region than in the periphery of the lobule (fig. 9). Fat droplets occur also in the blood in some preparations, in which case they lie intermixed with blood cells and give no indication of being carried there by the microtome knife.

Controlled diurnal cycle. The mice were fed from 7 to 10 P.M. since this seemed to be approximately the normal feeding time as revealed in the uncontrolled-feeding experiment.

The change of weight of the whole gut of the animal and of the stomach alone is shown in figure 2.

Cell size in the livers of the females rises for the first 9 hours following feeding, then slowly falls. It is very small in the livers of the males for the first 12 hours following feeding, rises to a sharp peak at 15 hours, then falls off steeply.

As for the mitochondria, in the livers of the males they are spheres and short rods at the periphery of the lobule and are quite heavy except at 21 and 24 hours after feeding; at the middle they are longer and somewhat finer rods, elongating to filamentous length toward the center. In the livers of the females, on the other hand, the chondriosomes are heavy spheres and short rods, except at 9 and 12 hours, when they are heavy filaments at the periphery of the lobule; at the middle they are filamentous at 3, 6, and 9 hours, and rods thereafter. At the center of the lobule, in both sexes, they are elongate, fine, and usually light filaments, sometimes fragmented near the central vein. There is a sharp line of demarcation between the cells of the middle zone, with their siderophilic mitochondria, and those of the central zone with their non-siderophilic ones.

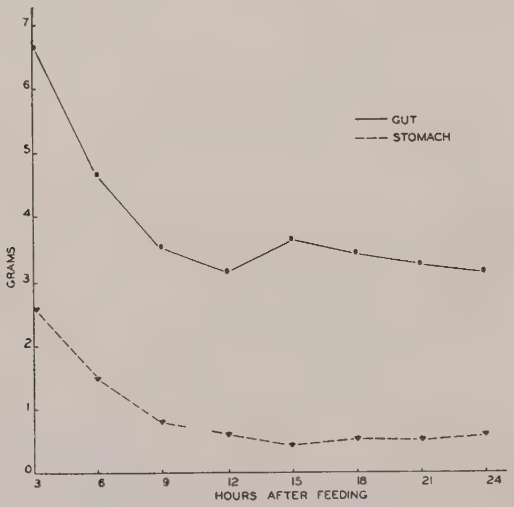

Fig. 2 Controlled feeding series: change in weight of gut and stomach during the 24-hour period.

A conspicuous cyclic feature of the mitochondrial preparations is the occurrence of a marked siderophily of the cytoplasm between the vacuole-like spaces during the hours after feeding (fig. 6). This occurs principally in the cells of the middle region of the lobule and only rarely in the cells adjacent to the portal and the central veins. The siderophily in the livers of the male animals is slight at 6 hours, then more marked until 18 hours, and disappears at 21 and 24 hours. The livers of the females exhibit a marked siderophily from 6 to 15 hours, then less, with none at 24 hours. As reported above, a rim of cells around the block of tissue shows neither the vacuolation nor the siderophily of the cytoplasm.

The Golgi substance is located in the form of networks in contact with the nucleus, in broken networks or isolated dictyosomes in the intermediate cytoplasm, and in continuous or discontinuous networks in contact with the surface of the bile capillary. The relative amount of the Golgi substance in these various positions, however, tends to shift slightly during the cycle, with more of it near the nucleus at 3 hours after feeding and more peripherally later in the day. There is likewise a cycle in the size of the Golgi substance and one in its osmiophily. The strands are very fine at 3 hours (fig. 7), become progressively heavier and more complex in their appearance until 18 hours (fig. 8), after which they again become single and more tenuous. Moreover the osmiophily of the Golgi bodies is high during the first 18 hours, then suddenly declines.

In addition to this variation of the Golgi substance in the series as a whole, there is also a variation in its size and osmiophily within the lobule. In the peripheral region it is composed of short and heavy strands, while in the more central region the networks tend to be looser and often more fragmented, and the strands appear much finer and less complex. In the rows of cells immediately around the central vein, the Golgi substance has a low degree of osmiophily.

The quantities of bile acid, glycogen, and fat in these livers are given in table 2.

The bile acids appear to be secreted continuously but in varying quantity. The granules are present in all cells of the lobule at all times: at 3 hours they are quite concentrated (fig. 14); they become more sparse at 6 hours, then gradually increase during the 24-hour period (fig. 15). In the early hours the granules are more numerous and possibly slightly larger in the peripheral zone than in the central; by 21 hours this distinction is not apparent.

Glycogen is already quite concentrated in the livers of animals killed 3 hours after the beginning of feeding. It increases in quantity until 15 hours in the males, until 9 hours in the

TABLE 2

Controlled diurnal cycle: bile acid, glycogen, and fat content of liver in histological preparations.

HOURS AFTER FEEDING	NO., SEX	BILE ACIDS	GLYCOGEN	FAT
3	1,♂	+++++	+++++	0
	2,♂	+++++	++++	0
	3,♀	+++++	+++	0
	4,♀	++++++	+++	0
6	1,♂	+++	+++++++	0
	2,♂	+++++	++++++	0
	3,♀	++	++++++	0
	4,♀	+++	++++++	0
9	1,♂	++++++	+++++	+
	2,♂	++++++	+++	0
	3,♀	+++++	++++++	0
	4,♀	++++++	+++++	0
12	1,♂	+++++	+++	0
	2,♂	++++++	+++	0
	3,♀	++++++	+++	++
	4,♀	+++++++	++++	+
15	1,♂	++++++	+++++	0
	2,♂	+++++++	++++++	(+)
	3,♀	+++++++	+++++	0
	4,♀	+++++++	+++++	0
18	1,♂	+++++++	++++	0
	2,♂	++++++	+++	0
	3,♀	+++++++	+++	+
	4,♀	+++++++	+++	0
21	1,♂	+++++++	+	0
	2,♂	++++++++	+++	0
	3,♀	+++++++++	(+)	0
	4,♀	+++++++++	++	0
24	1,♂	+++++	0	0
	2,♂	++++++	0	0
	3,♀	++++++	0	0
	4,♀	++++	0	0

females; at 21 hours it suddenly decreases in both sexes. In most of the preparations the glycogen is evenly distributed within the lobule (fig. 11); at 18 and 21 hours it may be more concentrated in the peripheral part of the lobule (fig. 12); and in one female at 21 hours and in one at 24 hours, it is more concentrated in the central part of the lobule.

Fat occurs in small droplets in the blood stream and within Kupffer cells in several preparations but very rarely in liver cells. In one male at 9 hours and in a female at 18 hours, fat droplets are present in the parenchymal cells of the central zone, and in the two females at 12 hours it is most concentrated in the cells of the peripheral part of the lobule.

DISCUSSION

A study has been made of the relation of the diurnal cycle of storage and secretion in the mammalian liver to the time of digestion and of the related cytological changes occurring in the hepatic cells.

The uncontrolled mouse exhibits a periodicity of eating, revealed by observations on the activity of the animals and by the weight changes of the gut and of the stomach during the 24-hour period (fig. 1). The animals normally feed for several hours in the late evening; there is also a second, shorter feeding period in the early morning. This description agrees essentially with those of Deuel et al. ('38) and of Pitts ('43) for the rat.

To determine whether storage does follow eating histological preparations demonstrating glycogen and fat were studied and calculations of cell size were made as well. Both Noël ('23) and Forsgren ('28, '35) have reported that an increase in cell size accompanies storage. But according to the results of my calculations not only may the cells be small when storing but they may also be distended when not containing any appreciable amount of glycogen. Consequently, although it would be reasonable to assume that a cell would be enlarged by the addition of glycogen to its cytoplasm, there must be so many complicating factors that the amount of storage cannot be ascertained by so simple a means as the calculation of cell size. This conclusion that there is no direct relationship between cell size and storage disagrees with that reached previously from calculations of cell size made on two preliminary series of animals with controlled feeding (Deane, '42b) where there was an apparent correlation. The Swiss mice suffered ill effects from the regimen: they lost considerable weight and certain individuals appeared weakened. The accompanying loss of water and possibly of solids from the cells may have been responsible for the small cell size during the storage period (McBride, '43).

With both controlled and uncontrolled feeding, glycogen appears to be deposited shortly after the time of eating. This observation agrees with the conclusions of Higgins, Berkson and Flock ('33) and of Deuel et al. ('38) that the glycogen deposited in the liver in the diurnal cycle is primarily alimentary in origin. There is no evidence to support the conclusions of Forsgren ('35) and of Sjögren et al. ('38) that the time of glycogen deposition is controlled by factors other than alimentation.

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My preparations show that glycogen is deposited initially in the peripheral zone of the lobule. This is in disagreement with the descriptions of most previous workers with the exception of Edlund and Holmgren ('40). In their preparations and in mine the glycogen is first deposited in the peripheral zone (fig. 10), then more centrally (fig. 11). When glycogenolysis sets in, a certain amount of glycogen disappears from the central region first (fig. 12), and all of it is then lost from the peripheral region, leaving a small quantity in the central region (fig. 13) before it disappears entirely (fig. 3). While Edlund and Holmgren believed that glycogenolysis begins first in the peripheral region and reaches the central region last, their data show that glycogen is sometimes more concentrated in the peripheral zone than in the central in the glycolytic period (their table 1), so that my suggested cycle could apply to their animals. The cycle proposed here may also explain the fact that some observers have declared that glycogen is more concentrated in the central zone of the lobule, while others have declared that it is more concentrated in the peripheral (e.g. Holmquist, '31, vs. Kater, '33, for the rat). Such a variation could result if animals were examined at different stages of the diurnal cycle.

The factors involved in this regular change in glycogen distribution have not been determined. The initial deposition of glycogen in the peripheral region may be due to the fact that the source of sugar is the blood, which enters by the portal vein. Glycogenolysis might begin in the central zone because of the relative anoxia of the region since, according to Mathews ('39, p. 1250), glycogen is immediately broken down to sugar under anaerobic conditions. But why a small amount remains in the central region to the very last is not clear.

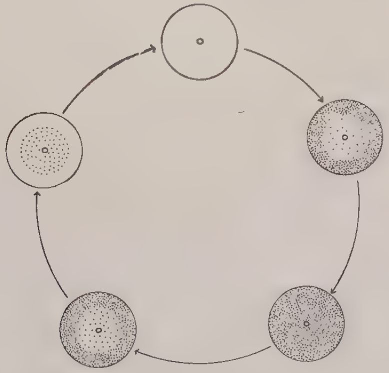

Fig. 3 Cycle of glycogen distribution in the liver lobule.

Whatever fat is present in the livers of the mice in these diurnal-cycle series seems also to be of alimentary origin, since it appears in the liver soon after the time of feeding. The fat droplets are more numerous in the central zone of the lobule in these animals, with the exception of the controlled-series, 12-hour females. It has been generally stated that fat is peripherally located in the healthy liver, but both Grafflin ('40) and Deane ('42a) described the deposition of fat in the center of the lobule under non-pathological conditions. A possible explanation may be that the peripheral cells, with their greater supply of oxygen, are better able than the central cells to oxidize or otherwise dispose of fat brought to them by the blood. This would result in the accumulation of fat droplets in the latter zone.

Although these experiments were not designed to determine whether or not there is an antagonism between the deposition of fat and of glycogen in the liver, as stated in the Rosenfeld ('02) rule, they give no support to the concept. Fat and glycogen are both deposited during alimentation and then both disappear with the subsequent fast. Likewise, fat and glycogen can be stored in a cell concomitantly, for, although it is not possible to demonstrate them both in the same preparation, the cells must contain both when they are present in all of the cells of a whole zone or of the whole lobule—for example, in the uncontrolled seres, 12 P.M. through 12 M.; in the controlled series, 9 hours, no. 1, 12 hours, nos. 3 and 4. Many other workers have also been unable to find any reciprocal relationship between the two substances, in either histological or biochemical studies (Noël, '23; Sünder, '37; Treadwell, Tidwell and Grafa, '43).

As for the secretion of bile acids, the preparations of both of the series show granules of fuchsinophil material in all of the cells of the liver lobule throughout the 24-hour period (figs. 14 and 15). The granules are more crowded and sometimes larger in the cells of the peripheral zone than in those of the central. Even though this observation agrees with that of Forsgren ('35) that secretion is most intense in the peripheral part of the lobule, the granules are not entirely limited to the peripheral region during maximal storage as he stated. But my observations support his statement that there is a periodic fluctuation in the quantity of bile acids secreted by the liver during the day. (In the ensuing discussion, "secretion" will be used to mean the intracellular formation of the product, and "excretion" to denote the passage of the product into the lumen—a terminology recommended by Bowen

('29).) The number of bile acid granules is lowest shortly after the time of eating, which may indicate an augmented excretion of bile following eating. And such an increase in bile flow had been observed in bile-fistula experiments by Kocour and Ivy ('38). Cellular secretion then rises gradually during the 24-hour period, the number of granules being greatest just prior to the expected feeding time.

From my observations, there does not appear to be a true inverse relationship between the storage of glycogen and the secretion of bile acids, such as Forsgren ('28, '35) has proposed. According to him, there is an antagonism in the two processes in the parenchymal cells, which results in an inverse relationship between them, not only in their amount but also in their distribution, glycogen being deposited first at the center of the lobule and bile being secreted continuously only by the most peripheral cells. In my preparations, secretion is shown to continue throughout the lobule even during maximal storage. Moreover the storage of glycogen as well as the secretion of bile acids is greatest in the periphery of the lobule. Although in the normal animal the peak of secretion occurs just before eating time and the peak of storage after, it is not necessary that the maxima occur at different times, for Jores ('40) has found that in hypophysectomized animals bile secretion and glycogen storage reach a peak at the same time. Therefore the same cells must be able to store glycogen and to secrete bile acids concomitantly.

The activities of the cell organelles in relation to the rhythm of secretion and of storage remain to be considered. The mitochondria in the controlled-feeding series exhibit the changes described by Noël ('23) in a similar experiment. After the feeding period, the mitochondria of the peripheral zone become shortened and somewhat swollen; those of the middle-zone cells are also shorter than normal. The changes in the uncontrolled-feeding series, however, differ from those in the controlled-feeding: the mitochondria are not shortened during the period of maximal glycogen content, but rather are elongate, becoming progressively shorter until the period of minimal glycogen content.

The question that presents itself here is whether the form of the mitochondrion is related to a specific function of the liver cell. Noël ('23) believed that rounded and enlarged mitochondria are an indication of storage by the liver cell, but the fact that a similar shortening of the mitochondria does not occur with the storage of glycogen in an uncontrolled diurnal cycle tends to refute this belief. Yet a rounding of the mitochondria of the peripheral-zone cells of the liver lobule, in the mouse at least, may be associated with activity that is more marked than usual. In the animals on controlled feeding the blood entering the liver must contain more of the products of digestion than is "normal", and this condition may serve to heighten the activity of the liver cells, particularly those at the periphery of the lobule. Indeed, the fact that the peripheral cells are always bathed with blood more highly oxygenated and more richly supplied with food-stuffs than the more central cells may be responsible for the shorter and broader mitochondria of the peripheral zone. The cytoplasm of the peripheral cells may also contain more of the materials which go into the formation of the mitochondria.

A study of the preparations leads one to feel that the mitochondria play no direct rôle in the processes of storage and secretion. Despite the conspicuous and quite constant zonation of the mitochondria, glycogen is most concentrated first in one zone, then in another; bile acids are secreted constantly in every cell in the lobule; fat comes and goes. There may be some significance, however, in the fact that the mitochondria are broadest in the periphery of the lobule where the secretion of bile acids is greatest. Both Berg ('20) and Noël ('23) have previously suggested that the mitochondria are associated with the secretion of bile acids. It is also true that, when fat is present in the central region of the lobule, the mitochondria of this zone are especially light and often fragmented in the row of cells adjacent to the central vein. Since the fat is also present outside this row of cells, however, the condition of the mitochondria does not suggest a direct relationship between it and the deposition of fat, but rather that the two conditions may result from the same cause—possibly the low oxygen tension in the central region of the lobule.

In each of the diurnal-cycle series there are livers that exhibit a marked siderophily of the cytoplasm: in the uncontrolled series, from 6 A.M. to 6 P.M. (fig. 5); in the controlled series, from 3 to 18 hours after feeding (fig. 6). The siderophily reaches its peak in those livers containing the greatest amount of glycogen; the same cells are filled with vacuole-like spaces. The occurrence of cytoplasmic siderophily has also been reported by Milletti ('37) and by Steffens ('41) at the time of a high glycogen content of the cells. This condition has sometimes been described as a clumping of the mitochondria, but a close study of the preparations indicated that it is rather a masking of the individual mitochondria by the darkened cytoplasm. In the siderophilic preparations, the whole cell may retain the stain or the condition may be localized in a part of the cell. The most intensely siderophilic cells are those in the middle zone; those next to the veins show the effect only when the siderophily is most intense. In all of these livers, the cells at the rim of the block are not vacuolated nor siderophilic. This observation suggests that the condition is an artifact somehow related to the speed of fixation: in the interior of the block, where fixation is delayed, the release of glycogen from its associated proteins, then its breakdown into diffusible sugars and their loss from the cell may be responsible for both the vacuolation of the cytoplasm and the modification of its staining reaction.

The Golgi substance in both the controlled- and the uncontrolled-feeding series is formed of loose, broken networks, lying near the nucleus and along the bile capillary, with fragments between. It lies typically between the nucleus and the excretory pole of the cell in exocrine gland cells (Bowen, '29; Kirkman and Severinghaus, '38).

The Golgi substance shows a diurnal rhythm in its location, in the breadth of its strands, and in the degree of osmic-acid reduction. It is more juxtanuclear after the eating period and more peripheral before. Also, after the feeding period the Golgi strands are black, fine, and contain small knots and vesicles (fig. 7); during the day, the strands become progressively thicker until 21 hours (fig. 8); they then become noticeably finer and less complex, and also less osmiophilic.

Cramer and Ludford ('26) proposed the following functional cycle of the Golgi apparatus of the hepatic cell: the Golgi net lies in a small compact knot next to the nucleus when the cell is at rest; it is fragmented in the intermediate cytoplasm and along the bile capillary in conditions of normal bile excretion; and it lies entirely peripherally, adjacent to the bile capillary, in maximal activity. The strands of the Golgi net, when juxtanuclear, are very fine, whereas the fragments lying along the bile capillary during maximal secretion are broad and complex in appearance and were called by the authors osmiophilic canaliculi. The Golgi apparatus in the liver cell is believed to participate in the secretion and ultimate excretion of the bile pigments.

In my preparations, the Golgi substance is never seen to lie either entirely juxtanuclearly or entirely peripherally in the cell. According to the Cramer-Ludford cycle, this observation indicates that the secretion of bile pigments is neither completely inhibited nor markedly stimulated during the 24-hour period. However, the fact that the bulk of the Golgi substance moves peripherally in the cytoplasm following the time of feeding may indicate that the secretion of bile pigments reaches a peak shortly before the expected feeding time as does that of the bile acids. Even more convincing in this respect is the simultaneous increase in the breadth of the strands, for Bowen ('29), Uhlenhuth ('34), and Kirkman and Severinghaus ('38) have all emphasized that an increase in the volume of the Golgi material and in the breadth of its strands always accompanies the formation of the secretion in gland cells.

As in the case of the mitochondria, the Golgi bodies are largest and darkest at the periphery of the lobule. This may also be related to the greater activity of these cells and to the greater supply of those materials that go into the formation of the Golgi substance.

The osmiophily of the Golgi apparatus is low not only at the center of the lobule but also throughout the lobule late in the diurnal cycle. The condition might be thought to result from a low oxygen tension or a low glycogen content of the cell, but the results of a supplementary experiment make such suggestions untenable. In animals fed a whole-egg diet, although a great deal of glycogen is deposited in the cells, the Golgi apparatus is not impregnated even after long treatment. Since egg contains very little carbohydrate (Needham, '27) it seems probable that most of this glycogen is derived from protein. Possibly, then, the Golgi material is less easily impregnated with osmic acid when the cell is carrying on gluconeogenesis.

In conclusion it may be said that the most conspicuous feature of the cytology of the liver is the zonation within the lobule in the changes in the form of the cell organelles and in the quantity of the substances that are stored and secreted, in relation to alimentation. The mitochondria are larger and shorter, the Golgi substance is heavier, bile secretion is greater, glycogen deposition is more marked, and fat storage is less marked at the periphery of the lobule than at the center. There appear to be two important environmental factors which might be influential in such zonation: one is the greater supply of oxygen at the periphery of the lobule; the other is the greater supply of foodstuffs. Although these two factors are essentially different, they act in an additive manner to make the peripheral cells the most active in the lobule. The heavier mitochondria and Golgi substance and the larger amount of glycogen and bile acids would all be manifestations of the high activity of the peripheral cells, while the accumulation of fat at the center of the lobule would reflect the lower activity here. Even though we have no clear evidence at present for any direct relationship between changes in the organelles and the processes of storage and secretion, there is therefore a certain parallelism in their cycles. This may be the reason why many other workers have believed that they were able to detect direct relationships between them. Furthermore, the facts that led Rosenfeld ('02) and Forsgren ('28, '35) to formulate their rules, which are quite inadequate to explain the observed distribution of substances in the liver lobule, can readily be accounted for by such a concept of the activity of the liver cell.

SUMMARY AND CONCLUSIONS

1. A study has been made of the relationship between changes in the mitochondria and the Golgi substance of the liver cell and in the processes of secretion of bile acids and storage of glycogen and of fat.
2. Mice were killed at 3-hour intervals during the 24-hour period, both with uncontrolled and controlled feeding.
3. Storage of glycogen occurs soon after the time of eating. There is not always a correlative increase in cell size.
4. Glycogen is laid down initially in the peripheral part of the lobule, then more centrally. During the period of glycogenolysis some disappears from the central region first, then all from the peripheral region, leaving a small quantity in the central region before it disappears entirely (fig. 3).
5. A small amount of fat is deposited in the central part of the lobule after eating. No antagonism was observed between the deposition of glycogen and that of fat.
6. Bile acid secretion reaches a peak shortly before the time of eating. It is most intense at the periphery of the lobule. No real antagonism exists between the secretion of bile acids and the storage of glycogen.
7. The mitochondria of the liver cells are always shorter, broader and more darkly staining at the periphery of the lobule than at the center. There appears to be no regular change in the mitochondria in relation to the diurnal cycle.
8. A cytoplasmic siderophily in mitochondrial preparations occurs when the cells are filled with glycogen and appears to be a fixation artifact.

9. The Golgi substance lies in networks beside the nucleus and along the bile capillary. More of it occurs in the peripheral position late in the diurnal cycle, when the strands are also broader and more osmiophilic.

10. The Golgi substance is larger and more osmiophilic in the peripheral part of the lobule than at the center.

11. There are no indications that either the mitochondria or the Golgi substance play direct rôles in the secretion of bile acids or in the storage of glycogen or fat. All related zonation phenomena are more probably dependent upon the quality of the blood bathing the cells.

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PLATES

EXPLANATION OF PHOTOMICROGRAPHS

The photographs were taken using apochromat objectives and compensating oculars. Figures 10, 11, 12 and 13 were taken with a 16-mm. (10×) objective, the rest with a 2-mm. oil immersion (90×) objective. The pictures of Heidenhain iron-alum hematoxylin preparations (for mitochondria) and of Champy-Kolatchev preparations (for the Golgi substance) were taken without any filter. Those of Forsgren preparations (for bile acids) were taken with a Wratten blue filter. Those of Bauer-Feulgen preparations (for glycogen) and of sudan IV preparations (for fat) were taken with a Wratten X_1 green filter.

PLATE 1

EXPLANATION OF FIGURES

4 Uncontrolled feeding experiment, 6 A.M. Regaud-Heidenhain preparation. Peripheral zone of the lobule during the storage phase: mitochondria heavy, crowded rods. Vacuolation of the cytoplasm. Magnification, × 1350.

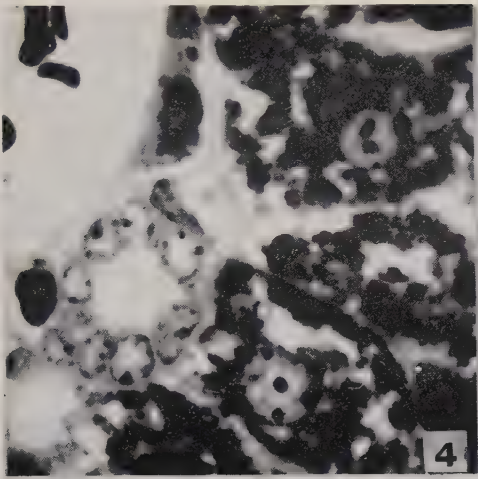
5 Uncontrolled feeding experiment, 6 A.M. Regaud-Heidenhain preparation. Middle zone to left, central zone to right. Storage phase: mitochondria fine beaded filaments; cytoplasm vacuolated and siderophilic in middle zone. Magnification, × 1350.

6 Controlled feeding experiment, 9 hours after feeding, no. 4,♀. Regaud-Heidenhain preparation. Peripheral zone of the lobule at height of glycogen storage; marked vacuolation and siderophily of the cytoplasm. Magnification, × 1350.

7 Controlled feeding experiment, 3 hours after feeding, no. 1,♂. Champy-Kolatchev preparation. Immediately after feeding, Golgi substance as fine osmiophilic strands near nuclei, in the intermediate cytoplasm, and along the bile capillaries. Magnification, × 1350.

8 Controlled feeding experiment, 18 hours after feeding, no. 1,♂. Champy-Kolatchev preparation. Late in diurnal cycle, Golgi substance as large, broad bodies located near nuclei and along bile capillaries. Magnification, × 1350.

9 Uncontrolled feeding experiment, 12 P.M. Sudan IV preparation. Fat droplets quite concentrated in central-zone cells. Magnification, × 760.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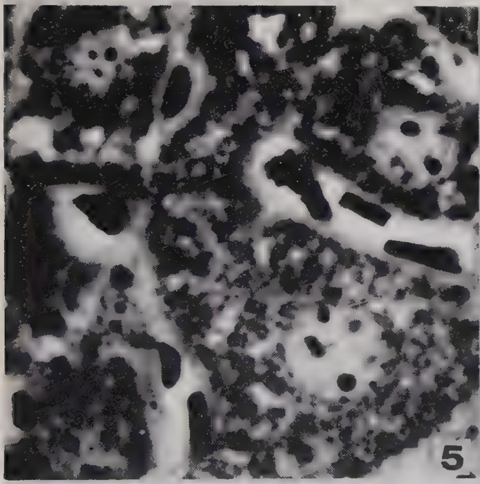
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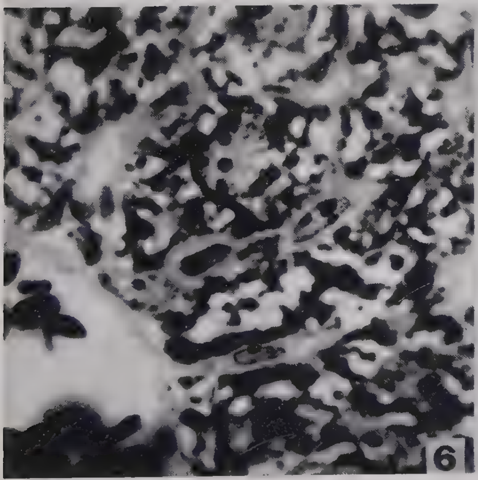
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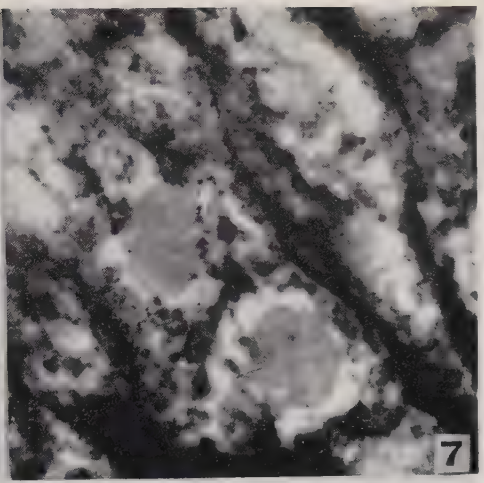
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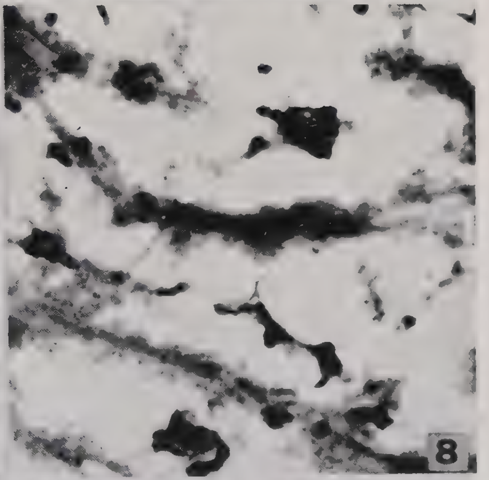
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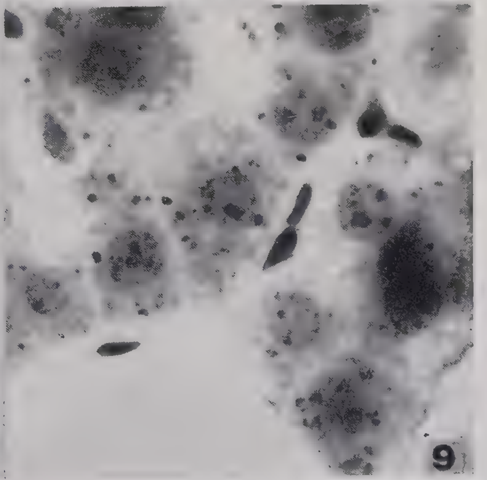
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PLATE 2

EXPLANATION OF FIGURES

10 Uncontrolled feeding experiment, 3 A.M. Bauer-Feulgen preparation. Glycogen being deposited, most concentrated in the periphery of the lobule. Magnification, × 110.

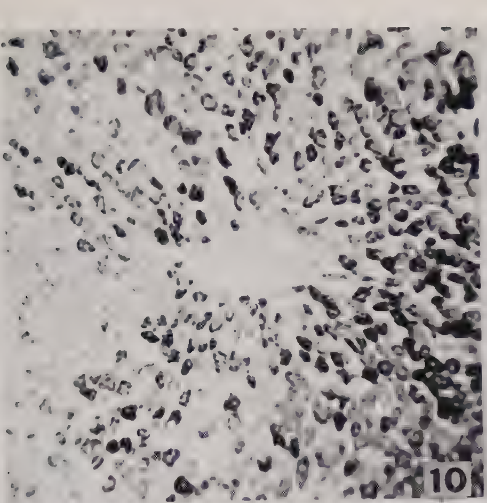
11 Controlled feeding experiment, 9 hours after feeding, no. 3,♀. Bauer-Feulgen preparation. Maximum deposition of glycogen, deposited evenly throughout the lobule. Magnification, × 110.

12 Controlled feeding experiment, 21 hours after feeding, no. 1,♂. Bauer-Feulgen preparation. First stage of glycogenolysis when larger deposit of glycogen is in the peripheral zone of the lobule. Magnification, × 110.

13 Uncontrolled feeding experiment, 6 P.M. Bauer-Feulgen preparation. Second stage of glycogenolysis when glycogen is only present in the central zone of the lobule. Magnification, × 110.

14 Controlled feeding experiment, 3 hours after feeding, no. 3,♀. Forsgren preparation. Middle-zone cells containing small and relatively sparse bile acid granules evenly dispersed in the cytoplasm. Magnification, × 1270.

15 Controlled feeding experiment, 21 hours after feeding, no. 4,♀. Forsgren preparation. Peripheral zone: large bile acid granules fill cells. Magnification, × 1350.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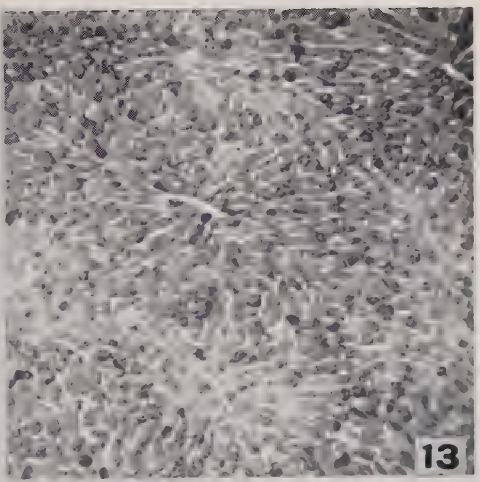
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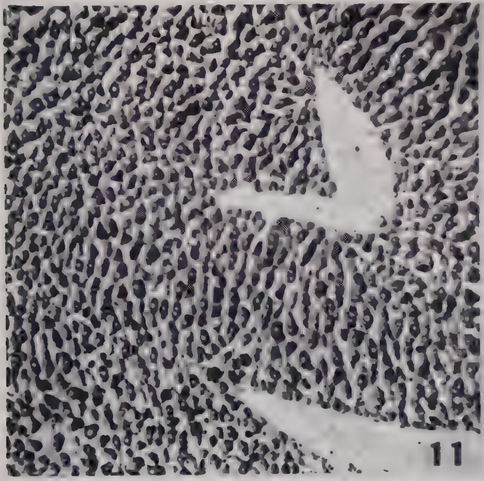
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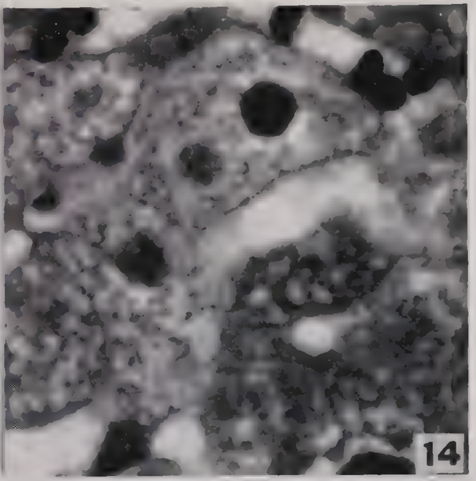
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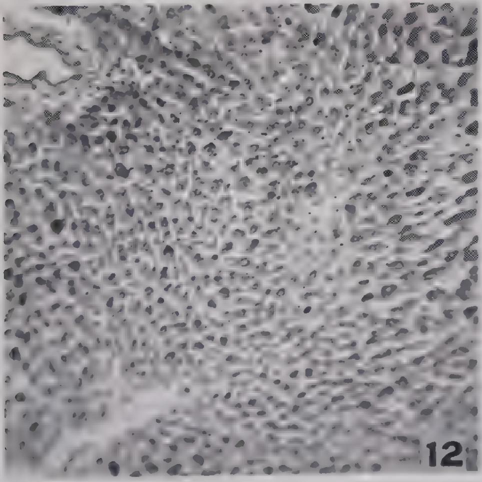
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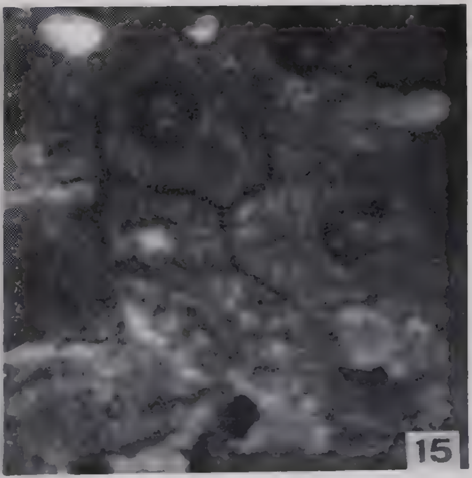
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STUDIES ON DEVELOPMENTAL PATHOLOGY

I. THE MORPHOGENESIS OF A HEREDITARY TYPE OF MICROPHTHALMIA IN CHICK EMBRYOS

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TWO PLATES (EIGHT FIGURES)

Hereditary microphthalmia was found in a stock of Barred Plymouth Rock chickens by Jeffrey ('41) who determined that the malformation is transmitted by a single autosomal recessive gene. He did not go beyond the genetic analysis of this anomaly, and gave no information on its nature beyond the facts that the eyes of the affected chickens are reduced in size to about one-half of the normal diameter, and that the chickens hatch, but die soon because they are blind and helpless. Thickening or doubling of the comb is associated with this microphthalmia. The present report deals with the morphology of the eyes of homozygous embryos from this stock.

A description of the morphogenesis of this hereditary malformation is being presented as the first in a series of investigations on developmental pathology not only because of the unique nature of the malformation itself, but also because it will be useful for the purpose of comparison with a non-hereditary type of microphthalmia in chick embryos to be described in a subsequent contribution.

MATERIAL AND METHOD

All embryos were fixed in Bouin's fluid, and received by the present author in alcohol. Some of them had previously been cleared in oil of wintergreen, and photographed with normal controls in oil and in alcohol. These embryos or parts of them, were embedded in paraffin, sectioned serially at 10 μ, and stained with the azan method.

The malformation becomes grossly visible at 6 days of incubation. In order to obtain earlier stages, younger embryos had to be sectioned at random. According to the transmission of the malformation by a single recessive gene, an incidence of 25% could be expected in the offspring of previously tested heterozygous birds. Embryos of 3½ and 5 days were examined in this manner. These as well as the older embryos used, are listed in table 1, together with the results of incubation in all eggs used.

OBSERVATIONS

In its fully developed form, and before the onset of degenerative changes, the type of michophthalmia under consideration is characterized by extensive folding of the optic cup (fig. 7). In the retinal area, only the inner layer is folded while the less conspicuous folds of the iris and ciliary region also comprise the outer layer. Many folds of the retina contain at their peaks closed cavities which do not communicate with the cleft in the fold itself, derived from the optic ventricle. The development of this condition, and its later changes will now be described chronologically.

Embryos of 3½ days. No indication of a malformation was found in sections of seven embryos.

Embryos of the fifth day. The embryos of this group varied considerably in size, and initial stages of the malformation were only found among the larger ones. This indicates that the changes in the eyes first appear at the end of the fifth day. There is no reduction of the size of the maldeveloped eyes at this early stage. The only abnormality consists of moderate thickenings of the retina (figs. 1 and 2) which appear singly or in groups. On central sections of these thickened areas a small lumen is seen, in its earliest stages not larger than the nucleus of an adjacent cell. Mitotic figures which are normally limited in the retina to the outermost layer of cells (nearest the pigment epithelium), appear in the cells lining these small cavities (fig. 2). A somewhat later stage in the development of the malformation is shown in figure 3. The cavities are increasing in size, thus causing the retina to bulge inward. Not all areas of maldevelopment in one embryo are at the same stage; figures 1 to 3, for instance, are taken from the same embryo. It could not be decided whether or not the separation of the retina from the

TABLE 1

TIME OF INCUBATION IN DAYS	STERILE EGGS	NORMAL EMBRYOS	MICROPHTHALMIC EMBRYOS	PARTS SECTIONED	DIAMETER OF ABNORMAL EYES	REMARKS
3½	1	7		whole embryos	(normal)	No malformation visible
5	?	17[1]	3[1]	heads	normal	Malformations found only in larger embryos of this group; some smaller ones may be homozygous but too young to show the malformation
6	4	2	2	abnormal heads	¾ of normal	
9	3	3	1	abnormal head		
10	3	1	1	abnormal head		
22	3	2 hatched 1 dead	1 alive failed to hatch	right eye region of abnormal embryo	½ of normal	

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[1] One each of these normal and homozygous embryos showed atypical unilateral microphthalmia. These will be described in a future report dealing with that type of microphthalmia.

pigment epithelium as shown in figure 3, is part of the malformation. It may be the beginning of the formation of one of the folds which are so prominent in later stages, and have the small cavities at their peaks (fig. 5). On the other hand, it is common experience that the two layers of the optic cup have a great tendency to separate, post mortem, so that the elevation of the retina in question cannot be definitely evaluated.

In brief, the changes in embryos of the fifth day consist of abnormally directed growth of the retina with the appearance of new growth centers around small cavities between the cells. These cavities are from their earliest stages separated from the potential optic ventricle which is normally lined by dividing cells.

Embryos of 6 days. At this stage the eyes of the homozygous embryos have become appreciably undersized. Comparison with a normal embryo (fig. 4) indicates that the diameter of the eye, as measured in this photograph and in sections, has been reduced to approximately three-fourths normal size.

Microscopically, the most prominent feature at this stage are steep folds of the retina projecting into the vitreous body. Many of these contain at their peaks the cysts described in the 5-day embryos (fig. 5). These cysts have grown, but their cavities have remained separated from the optic ventricle extending into the folds, as can be seen in serial sections. Not all folds contain these cysts, and, on the other hand, small and apparently newly formed cysts are present in slight bulges of the retina, not associated with folds. They are very similar to those seen in 5-day embryos, except for their location somewhat nearer the inner surface of the retina. It is thus obvious that cysts originate during a long period of development, so that various stages in their formation and association with folds are seen in the same eye. Large numbers of small folds are now present in the pars iridica retinae. The pigment epithelium does not participate in the formation of any of the folds found at this stage. The lips of the chorioid fissure are slightly drawn in, so that they look like some of the other retinal folds at the first glance. All other parts of the eye appear normal.

Embryo of 9 days. A comparison of normal and microphthalmic embryos of this age shows that the relative difference in the size of the eyes has increased (fig. 6). On section (fig. 7) numerous large folds are seen in the retina, many of them arranged in groups, particularly near the iris. The inner layer of the iris itself is thrown in folds throughout. There is no more evidence of new formation of cysts anywhere in the retina, and all existing cysts are now asociated with folds. The larger folds show the beginning of disintegration at their peaks. This condition is identical with that described by Gayer ('42) in retinal folds of abnormal eyes of different character. Gayer plausibly explains the degeneration by a deficiency of the nutrition in these areas far distant from the chorioid membrane with its blood vessels. The pigment epithelium is not folded except in the iris region where the folding somewhat exceeds the normal extent. No normal pecten is present, but only a short, club-shaped mass protruding inward from the chorioid fissure. Large numbers of thin-walled blood vessels are now found in the vitreous body near the chorioid fissure, and extending toward the lens. These vessels were not seen in any of the embryos of 5 or 6 days. Numerous isolated stellate cells are present in the vitreous body near the vessels. Compared with the 6-day stage, the retina of the 9-day embryo shows no new progressive changes toward the abnormal. This latter period is characterized chiefly by degenerative changes in the abnormal parts of the retina.

Embryo of 10 days. The condition of the eyes of this embryo is similar to that in the 9-day specimen. The degeneration of the folds has made some progress. The pecten is again almost absent, only a small cell mass attached to the lips of the chorioid fissure might be interpreted as its vestige. Blood vessels reach from the fissure into the vitreous body as in the 9-day embryo.

Embryo of 22 days. Measurements of the diameter of the eye on sections show that it is approximately one half that of normal eyes, which is in agreement with Jeffrey's data. For technical reasons, this large eye also had to be embedded in paraffin rather than celloidin, so that some of the gross distortions of its layers as seen in figure 8, may be artefacts.

The flat portions of the retina have developed their typical layers. The degeneration of the retinal folds is far advanced. No layers can be distinguished in them, and only a few nuclei are stained. Iris and ciliary body form a common thick mass mass around the equator of the lens, consisting of irregularly folded pigmented and unpigmented layers. In the region of the chorioid fissure the retina is considerably thickened by a large non-cellular layer, probably containing mostly nerve fibers in an irregular arrangement. A solid mass containing many blood vessels and pigmented cells protrudes from this region into the vitreous body. It is probably a derivative of the cell mass which was seen in embryos of 9 and 10 days in the place of the pecten. Only in a small area, pigmented folds resembling pecten structure may be seen in the eye of the present embryo. Thin-walled blood vessels are again found in the vitreous body in this region. The optic nerve is markedly thinner than normal, its diameter being about one-half that of a normal nerve.

The scleral cartilage is definitely thickened. Both its thickest and thinnest portions are twice as thick as those of a normal eye of the same age. This is in agreement with the results of Weiss and Amprino ('40) who found experimentally that a reduction of the tension of the growing eye results in a thickening of the scleral cartilage. In the present malformation the eye grew only to a fraction of its normal size, so that its growth pressure upon the sclera was below normal.

The lens of the abnormal eye is only slightly smaller than normal. Exact measurements on sections are impossible because of the damage in the process of sectioning. It can be concluded that the maldeveloped chickens are blind not only because of the anomaly of the retina (fig. 8 shows that large areas are normal), but also because of refractive anomalies due to abnormal relative size and position of the refractive media and the malformation of iris and ciliary body.

DISCUSSION

The primary morphological anomaly in the present hereditary microphthalmia is the establishment of abnormal growth centers in the retina around small cysts. This happens during the fifth day, at a time when the eyes are still of normal size, and structurally otherwise normal. From that stage on the growth of the eyeball lags until, at the end of incubation, its diameter is only about one-half of normal. During this period of reduced over-all growth steep folds appear in the retina, while the formation of new cysts continues. The cysts are soon found on the peaks of retinal folds, but other folds develop independent of cysts. Six-day embryos show these progressive changes, whereas the next stage examined, of 9 days, shows no indication of further new formation of cysts or folds in the retina. The previously developed folds with or without cysts begin to degenerate, probably as a consequence of insufficient nutrition. This degenerative phase continues through the rest of the incubation period. The pigment epithelium does not participate in the folding except in the region of iris and ciliary body where both layers of the optic cup form masses of small irregular folds.

Weiss and Amprino ('40) produced microphthalmia with a folded retina by puncturing the eyes of normal 4-day chick embryos. These eyes collapsed and remained small, apparently because the vitreous body had escaped. The fact that the retina was folded in both these experimental and the present genetically abnormal embryos, suggests a tendency on the part of that layer to develop to its normal size regardless of the available space. The pigment epithelium, on the other hand, evenly lines the eyeball regardless of its size in both types. In spite of these similarities, the mechanism of development is fundamentally different in these two kinds of malformation. In the case of experimental puncture, a presumptive normal retina is forced to fold by mechanical action, while in the hereditary microphthalmia the retina undergoes cyst formation without a comparable mechanical cause. The manner in which the cysts are formed, suggests in no way a response to the action of mechanical forces tending to reduce the size of the eyeball. Whether the following folding is part of the primary process, or secondary to it, cannot be decided. What little can be seen in sections of the vitreous body, does not suggest a deficiency on its part.

If the formation of cysts and abnormal growth centers within the retina is here described as the primary change, it must be remembered that this refers only to the chain of events detectable by purely morphological methods. Experiments with the maldeveloped eyes of homozygous Creeper chick embryos (Gayer, '42; Gayer and Hamburger, '43) have revealed that entirely unexpected and invisible actions of surrounding parts may determine a malformation in a potentially normal optic cup, and this may hold for the anomaly under consideration as well.

In classifying the present type of microphthalmia, the criteria may be used which v. Szily accepted in his work on eye malformations. In regard to the causation and transmission of the anomaly, v. Szily advocates the distinction of idiotypic and paratypic malformations. The former are inherited in identical or related forms while the latter are not. As to the ontogenesis, v. Szily distinguishes typical and atypical malformations. Typical anomalies go through a series of characteristic and regular developmental stages, so that their morphogenesis can be determined just like that of a normal organ. Atypical malformations, on the other hand, show no such regularity in their development or conformity in their structure, and appear pathological from the beginning. These two kinds of classification match in such a manner that the idiotypic malformations are typical in their development, and the paratypic ones atypical. This applies well to the present microphthalmia. According to its causation it is idiotypic, and in regard to its development it is a typical malformation. In the latter respect it is an instructive example because the primary morphological anomaly in its development is not a quantitative modification of normal development such as retardation or exaggeration, but a new process. This new kind of developmental process is in its course just as regular as a normal one, which identifies it as a typical malformation.

To the best of the author's knowledge no comparable malformation has been described in human or animal pathology. Many hereditary eye malformations are on record (Howe, '28), and microphthalmia is common among them, but it is the expression of a variety of developmental disturbances none of which are known to be primarily similar to the one described here. However, it must be remembered that the final condition of the present malformation is an uucharacteristic microphthalmia and gives no indication of the peculiar initial change. Similarly, the observation of hereditary or sporadic cases cannot very well reveal their nature and possible similarity with the malformation under consideration unless early stages are examined.

SUMMARY

The morphogenesis of a hereditary microphthalmia in chick embryos is described. In accordance with the genetic causation, a regular succession of stages was found, leading to uniform conditions in all embryos of comparable age.

The initial change is the formation of minute cysts in the retina. The cells lining these cavities divide mitotically and thus form abnormal growth centers. Soon folds appear and the cysts are then found on the peaks of some of them. Later on the folds, and with them the cysts, show signs of degeneration, apparently because of their great distance from the blood supply of the chorioid membrane. Iris and ciliary body also show abnormal folds of their ectodermal layers.

The pecten is almost absent, its greater part being replaced by a solid mass of tissue. The scleral cartilage is thicker than normal. The lens is only slightly reduced in size while the diameter of the eyeball itelf is finally, at the end of incubation, reduced to one-half of normal. The significance of these findings is discussed.

ADDENDUM

After completion of this report it was found that the walls of the cerebral hemispheres of the 10-day embryo contain a few small cavities lined by ependyma with dividing cells. These cysts closely resemble the initial changes in the retina of 5-day embryos. One similar cavity was found in the 22-day embryo. No comparable changes could be seen in any of the younger specimens. In view of the small number of observations this condition will not be discussed now. However, it is hoped that additional material will be available for further study of the brain.

ACKNOWLEDGMENT

The present work was made possible through the courtesy of Drs. Viktor Hamburger and Kenneth Gayer of Washington University who furnished all embryos as well as photographs of whole specimens from the stock previously examined by Jeffrey.

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PLATE

PLATE 1

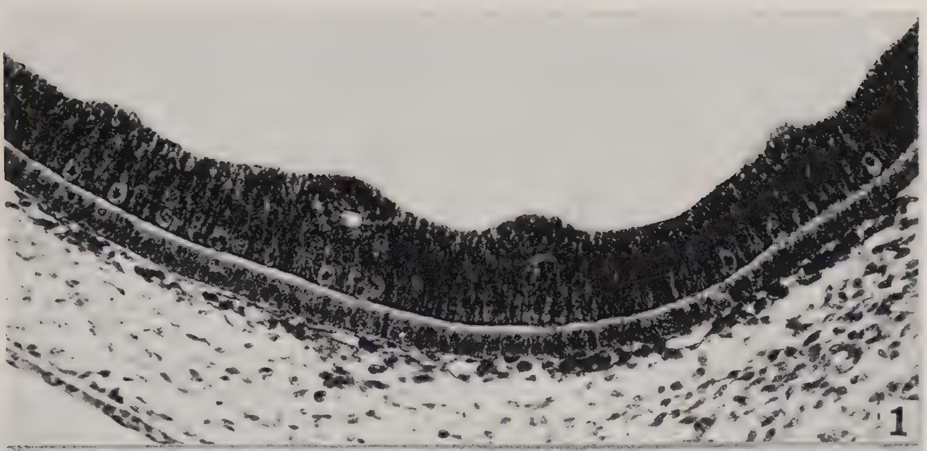
EXPLANATION OF FIGURES

Early changes in the retina of a 5-day embryo.

1 A group of slight thickenings of the retina. One of them is cut centrally, and a small cavity is visible in it.

2 Two bulges of the retina similar to those of figure 1, under high magnification. In one of them, the cavity is shown with a mitosis of one of the cells lining it.

3 A group of cavities in the retina, somewhat further advanced in development.



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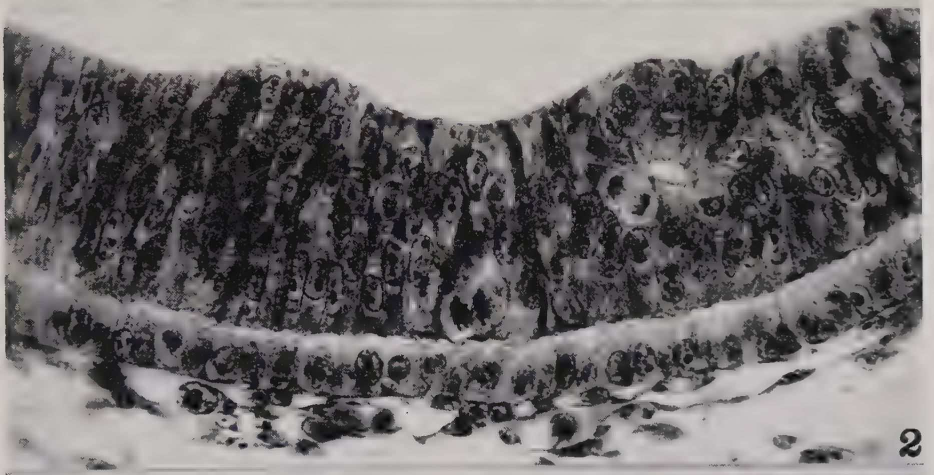

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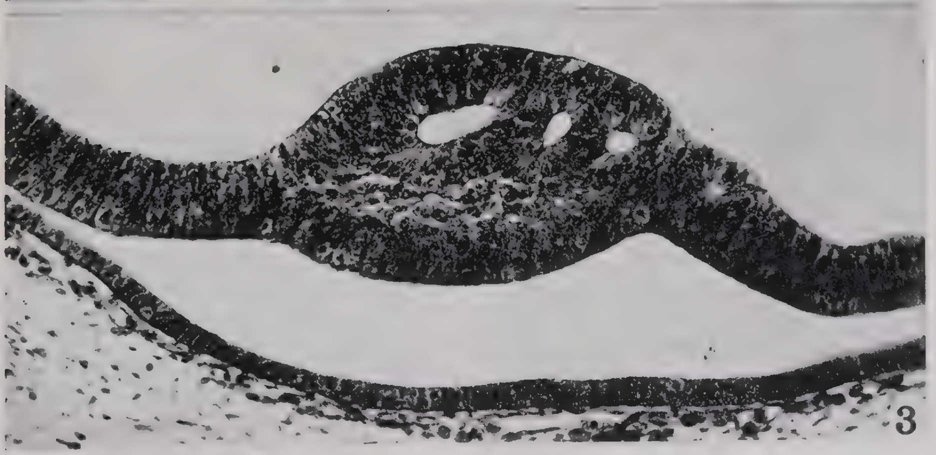

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PLATE 2

EXPLANATION OF FIGURES

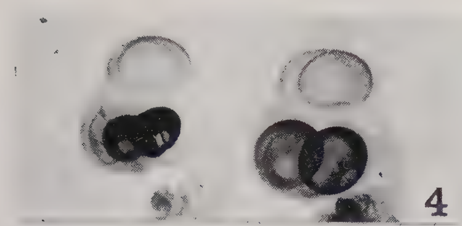
4 Cleared heads of a microphthalmic and a normal embryo of 6 days.

5 A fold in the retina of a microphthalmic embryo of 6 days. Serial sections show that the cavity at its peak is completely separated from the cleft in the fold.

6 Cleared heads of a microphthalmic and a normal embryo of 9 days.

7 Section of the eyes of a microphthalmic embryo of 9 days.

8 Section of an eye of a 22-day microphthalmic embryo.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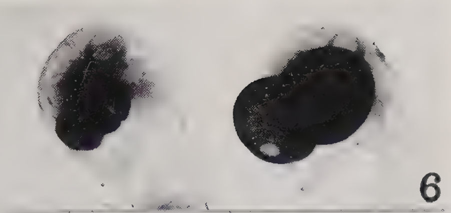
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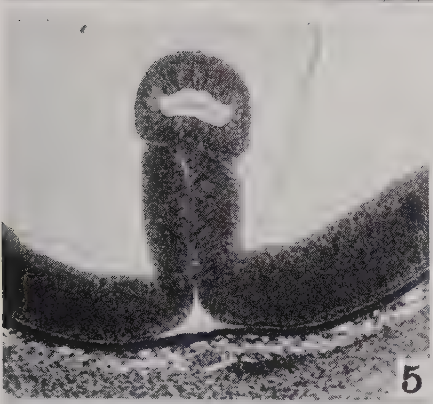
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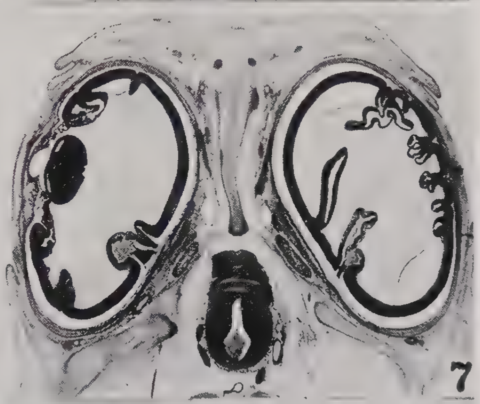
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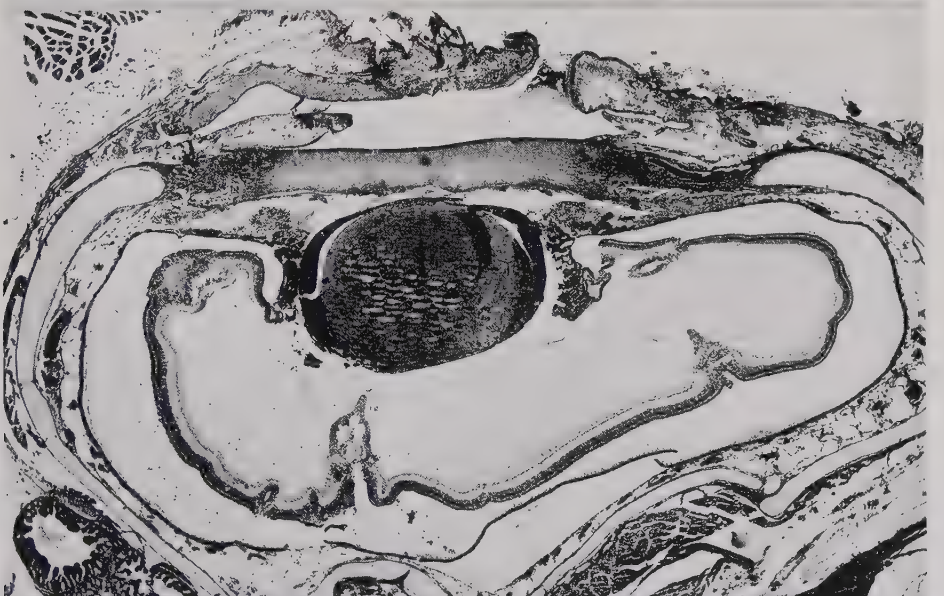
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STORAGE OF TRYPAN BLUE IN THE INTERNAL EAR OF THE RAT[1]

BENJAMIN SPECTOR
Department of Anatomy, Tufts College Medical School, Boston, Massachusetts

TWO PLATES (FOUR FIGURES)

There have been few attempts to study the presence of phagocytes in the organ of Corti and its associated periotic spaces by means of intra-vitam staining. It is of special interest, therefore, to learn something of the distribution of such cells in the internal ear. It is well established that the histiocytes of most all tissues possess in pre-eminent degree the capacity for storing in their cytoplasm the particles of a colloidal solution of trypan blue. The following brief report gives experimental evidence of the fact that the histiocytes in the internal ear store particles of this dye in their cytoplasm.

MATERIAL AND METHODS

Ten albino rats (Wistar strain) ranging in age from 12 to 45 days were given daily injections of 1 cc. of a 1% solution of the colloidal dye trypan blue. The solution, after being filtered and sterilized, was injected into the animals by the intra-abdominal route. The animals were killed at intervals from 24 to 68 hours and the otic capsules immediately fixed in Heidenhain's "Susa" mixture. The specimens were decalcified in 5% aqueous solution of trichloracetic acid and embedded in paraffin. The blocks of tissue were cut serially at 10 micra and stained with Mayer's paracarmine and orange G.

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[1] Aided by a grant from the Charlton Research Fund, Tufts College Medical School.

OBSERVATIONS

A careful examination of the sections of the experimental otic capsules revealed the presence of large oval or round cells and occasional elongated spindle shaped cells containing granules of trypan blue in the form of dust-like particles, small discrete granules or larger masses of agglutinated granules. The distribution of these histiocytes was extensive. There were abundant macrophages with prominent dye inclusions concentrated around the foot of the stapes and the tympanic membrane. The scala tympani and the scala vestibuli showed many of these cells applied to their walls. Within the modiolus and about the nerve fibers numerous histiocytes filled with blue granules of varying size were clearly in evidence. A few spindle shaped cells were observed lining the basilar membrane. An interesting observation was the presence of a large histiocyte, laden with dye, within the ''tunnel'' of the spiral organ. One also observed many histiocytes intensely stained with the vital dye about the semicircular ducts. There was no evidence of devitalized areas of protoplasm. A careful search revealed no cells laden with dye particles as part of a reticulum. All of the cells were free macrophages. The accompanying colored figures clearly illustrate the distribution of the histiocytes in selected areas of the internal ear.

SUMMARY

1. Following intra-abdominal injections of trypan blue in albino rats, the histiocytes in the internal ear react to the storage of this dye in a manner characteristic of histiocytes found in most all tissues.

2. The storage of the transported dye was found in the histiocytes lining the periotic spaces, the basilar membrane, and the tympanic membrane. In addition, the blue granules were stored in the histiocytes about the stapes, the modiolus, the nerve fibers and the semicircular ducts.

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PLATE 1

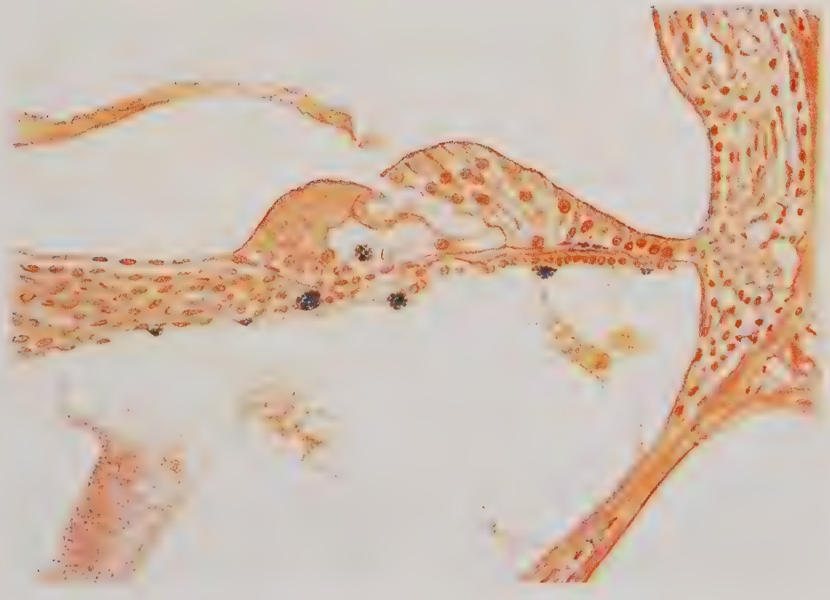
EXPLANATION OF FIGURES

1 Note: the large histiocyte in the ''tunnel'' of the spiral organ; the elongated cells at the basilar membrane; the macrophages lining the scala tympani. × 280.

2 Shows the detail in the storage of the dye. × 1450.

TRYPAN BLUE IN INTERNAL EAR OF RAT
BENJAMIN SPECTOR

PLATE 1



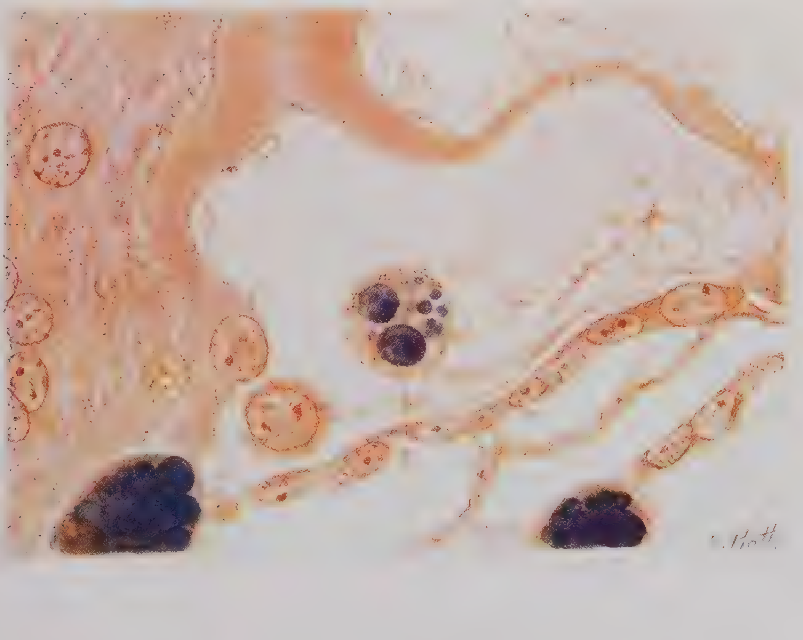
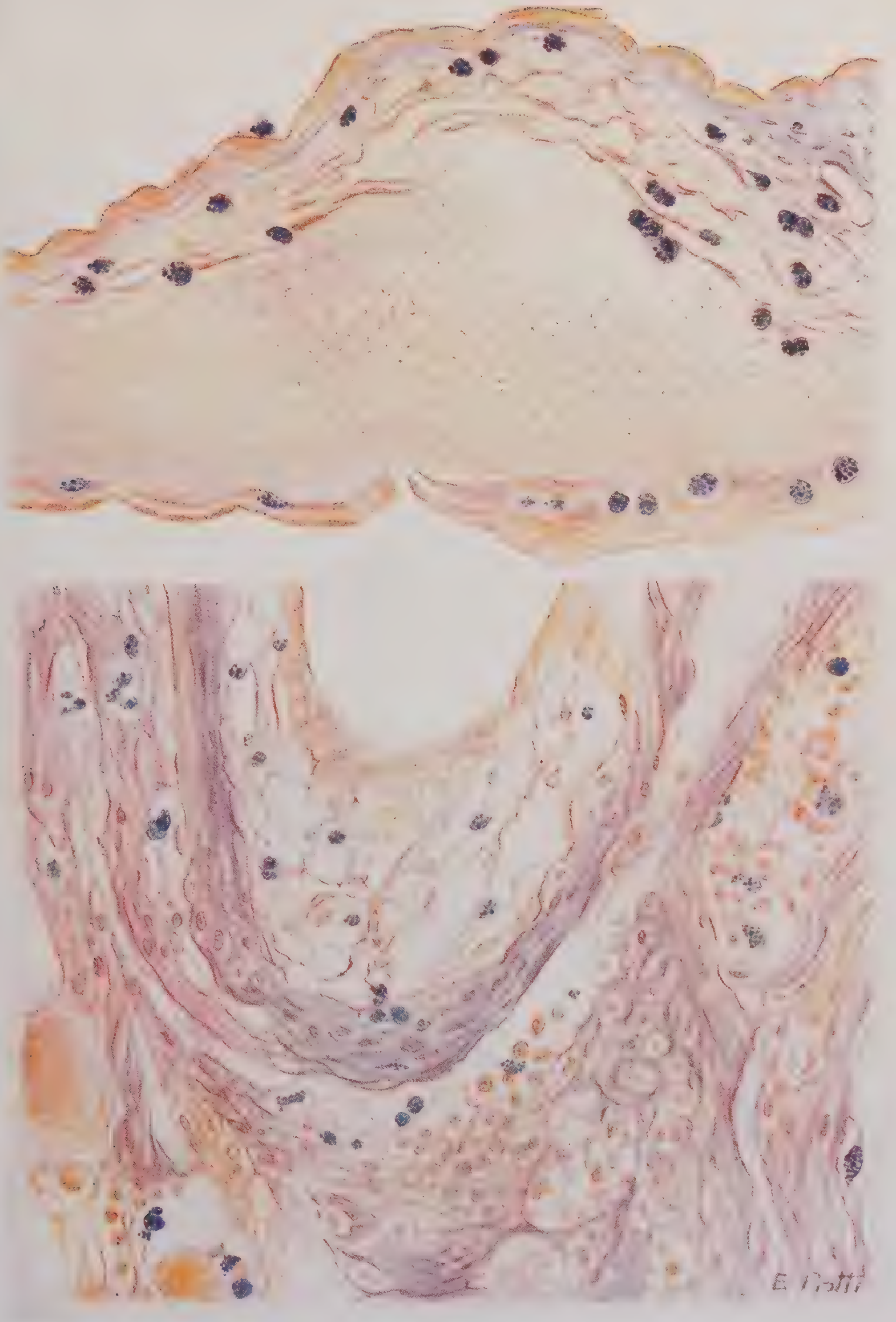


PLATE 2

EXPLANATION OF FIGURES

3 Portion of the tympanic membrane showing histiocytes laden with particles of trypan blue. × 500.

4 Section through the wall of the lateral semicircular duct and its surrounding tissue. Note the character of the dye inclusions in the histiocytes. × 450.


E. Gatti

THE DEVELOPMENTAL ANATOMY OF THE HUMAN OSSEOUS SKELETON DURING THE EMBRYONIC, FETAL AND CIRCUMNATAL PERIODS[1]

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FOUR PLATES (EIGHT FIGURES)

INTRODUCTION

The literature of the developmental anatomy of the gross human prenatal osseous skeleton has been primarily concerned with the time of appearance, the number and location of the ossification centers, the determination of fetal age on the basis of the presence and absence of specific centers, the interpretation of osseous anomalies, the development of the individual bones, and the relation of certain extrinsic and intrinsic factors to skeletal development. A few systematic researches have been made presenting pictorial and verbal descriptions of the general morphological development of the osseous skeleton as a unit (Kerkring, 1670, 1717; Albinus, 1737; Rambaud and Renault, 1864; Lambertz, '00; Alexander, '06; Mall, '06; Hess, '17; Dorland and Hubeny, '26, and others). No worker has figured the development of the prenatal skeleton in a series of orthoscopic drawings held to some constant dimension.

With this in mind, the purpose of this paper is to present, first, a pictorial and a brief verbal description of the development of the human skeleton up to and including the time of birth, by seven stages based on the examination of a series of stained transparent embryos and fetuses; second, some observations of the developmental anatomy of some of the individual bones; and third, some general concepts pertaining to the prenatal skeleton. No attempt is made to assay the determination of fetal age by fetal osteology.

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[1] Portion of the thesis submitted to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements of the degree of Doctor of Philosophy.

[2] Manuscript prepared at the University of Georgia School of Medicine, Augusta, Georgia.

For his advice, aid and counsel during the entire progress of this problem, I wish to express my sincerest thanks to Dr. Richard E. Scammon. I am indebted to Mr. Lawrence Benson for the precise execution of the series of skeletal illustrations and to the Department of Anatomy, University of Minnesota, for the facilities and materials supplied for this research.

HISTORY

From the time of Galen (ca. 130–200 A.D.) to the present, the gross developmental anatomy of the human prenatal osseous skeleton has been the object of much research. In a general way the bibliography of this field may be classified as: (1) literature concerned with the problems of the various aspects of the development of a single bone or a group of bones, and (2) literature concerned with the problems related to the development of the skeleton as a whole.

Most of the literature may be placed in the first group. Galen, who described seven ossification centers in the ossifying sternum and two centers for the mandible, and Fallopius (1561), who noted five centers in the prenatal sphenoid bone, three centers in each vertebra, four centers in the axis and eight centers in the newborn sternum, are examples of the pioneer workers. Weber (1830), and Rambaud and Renault (1864), presented excellent reviews and bibliographies of the work in this period. Descriptions of the ossification and development of the sphenoid bone by Huxley (1864), of the mandible by Low ('09), of the diaphyses of the long bones by Horand ('08), of the clavicle by Hanson ('20) and of the petrosal bone by Bast ('30) are examples of the literature since the time of Rambaud and Renault (1864). Von Spee

(1896), Bardeen ('10), Bryce ('15), deBeer ('37) and other references are bibliographical sources of this phase of the literature. The problems of the number and location of the ossification centers of each bone, of the prenatal developmental anatomy of each individual bone, and of the interpretation of congenital anomalies have been the primary topics of this literature.

The main topics of the literature of the second group are: (1) the attempt to establish the state of skeletal maturation as a criterion for the determination of fetal age and term (Hess, '17; Hill, '39 and others); (2) the developmental anatomy of the skeleton as a unit (Kerkring, 1670; Mall, '06 and others); and (3) the determination and evaluation of the factors (intrinsic: such as endocrine, hereditary and parity; and extrinsic: such as the nutritional and mechanical) influencing the appearance of the ossification centers and the growth of the bones (Noback, '43, for examples of this literature).

The classical researches of Kerkring (1670, 1717), Albinus (1737) and Rambaud and Renault (1864) present excellent original accounts of the ossification of the prenatal and circumnatal skeleton. Detailed, accurate and proportional drawings in these papers illustrate the skeleton and its parts. The analysis of the development of the prenatal and circumnatal skeleton from roentgenograms has been the subject of numerous papers from the pioneer researches of Bade ('00) and Lambertz ('00) to the recent work of Flecker ('42) and others. Mall ('06) appears to be the only worker to utilize either the clearing or clearing and staining techniques to analyze some aspects of the development of the entire skeleton during even a portion of prenatal life.

For further bibliography of the literature of the developmental anatomy of the human osseous skeleton during the embryonic, fetal and circumnatal periods, see Noback ('43).

MATERIALS AND METHODS

A series of forty human embryos, fetuses and a newborn infant, distributed throughout the period from the 23 mm. CR stage to birth, were cleared with potassium hydroxide and their osseous skeletons were stained with alizarin red S by a technique described by Noback ('43) and Noback and Noback (unpublished). These specimens were distributed as follows: 2 embryos in the second lunar month, 12 fetuses in the third lunar month, 8 fetuses in the fourth lunar month, 5 fetuses in the fifth lunar month, 4 fetuses in the sixth lunar month, 4 fetuses in the seventh lunar month, 1 fetus in the eighth lunar month, 2 fetuses in the ninth lunar month, 1 fetus in the tenth lunar month and 1 newborn infant. In addition, 10 other late fetal and newborn infant skulls were dissected.

From the cleared and stained series, seven specimens were selected and were used to illustrate a series of seven stages (figs. 2–8). An anterior view, a posterior view, and a lateral view of each specimen was drawn. All drawings were made to constant crown rump length.

The technique for making the drawings of the specimens selected to illustrate the stages consisted of the following five steps:

1. The specimen, floating in glycerin, was pinned down as desired to a paraffin base. Three views (anterior, posterior and lateral) of each specimen were photographed with a mounted 35-mm. camera.
2. The 35-mm. negative was then enlarged on a projecting machine to the predetermined CR length selected for all drawings, and a preliminary sketch was made.
3. The preliminary sketch was retouched freehand to add minor details observed directly from the specimen.
4. The retouched sketch was transferred to Ross board.
5. The Ross board drawing was finished in detail by observation of the specimen.

By this method it is felt that the illustrations were proportional and that they were drawn with a negligible amount of distortion.

SEVEN STAGES IN THE DEVELOPMENT OF THE OSSEOUS SKELETON UP TO AND INCLUDING THE CIRCUMNATAL PERIOD

The development of the prenatal and the circumnatal osseous skeleton is briefly described and illustrated (figs. 2–8) in a series of seven stages. The rate of differentiation of the skeleton determined the length of the intervals between the stages. When differentiation is rapid there is a short time interval between two stages, as 14 days between stage I (fig. 2) and stage II (fig. 3). When differentiation is slow there is a long time interval between two stages, as 5 months between stage VI (fig. 7) and stage VII (fig. 8). The drawings illustrating these stages were made from cleared and stained specimens that were selected as being representative for these stages. Variations, which are frequent in the developing skeleton, are illustrated in some of the figures. For example, in stage IV (fig. 5) an enlarged frontal and metopic fontellar area is shown in the anterior view and a Kerkring's ossicle, rarely present, is illustrated in the posterior view. The seven stages follow.

STAGE NUMBER	CROWN RUMP LENGTH	CROWN HEEL LENGTH	PROBABLE AGES [1]
	mm.	*mm.*	
I	38		64 days
II	56	75	78 days
III	88	125	3.25 lunar months
IV	105	156	3.5 lunar months
V	139	205	4.25 lunar months
VI	175	255	5 lunar months
VII	352	520	10 lunar months

[1] Probable age determined from the linear dimensions (CR and CH length) from formulae computed by Scammon and Calkins ('23), Scammon ('37) and Boyd ('41).

Stage I (fig. 2)

Length, 38 mm. CR. Age, 64 days (approximately).

Bones present —

Facial bones: mandible, maxilla, premaxilla, nasal bone, vomer, palate bone, zygomatic bone and internal pterygoid plate.

Calvarial bones: frontal bone, parietal bone, interparietal bone. supraoccipital bone, squamosal bone and tympanic annulus.

Basicranial bones: exoccipital bone and great wing (alisphenoid bone) of the sphenoid bone.

Thoracic bones: clavicle, scapula, and all ribs.

Vertebral column bones: cervical arches 3, 4, 5, 6 and 7, and thoracic arches 1 and 3 present on the right side; and cervical arches 3 and 5 and thoracic arches 1 and 2 present on the left side.

Upper extremity bones: diaphyses of the humerus, ulna, radius, metacarpal 2, 3, and 4, and all terminal phalanges.

Pelvic bones: ilium.

Lower extremity bones: diaphyses of the femur, tibia, fibula, metatarsals 2 and 3, and terminal phalanx 1.

Description —

The differences in the amount of differentiation among the facial bones, the calvarial bones, and the basicranial bones are the most striking features in the skull at this stage. The facial bones, except for the lacrimal bone which is absent in the specimen drawn, are all present and are more fully differentiated than the calvarial bones which are all present. Few of the basicranial centers have appeared. Each facial bone is characterized by the same general features as the corresponding adult bone. The facial sutural spaces are broad when compared to their definitive size but they are relatively narrow when compared to the broad sutural gaps of the calvarial bones. The calvarial bones are relatively small, rapidly differentiating bones with broad membranous areas separating them from each other. Except for the small ossification centers present, the basicranium is essentially cartilage.

The maxilla has well developed frontal, alveolar, zygomatic, palatine and orbital processes. The sutura notha is indicated by small unossified intervals at the premaxillary junction especially superiorly and on the palatine process. The interpremaxillary suture is rather wide and the infraorbital canal is a groove in the orbital facies. Characteristically the mandible is depressed as it is throughout prenatal life. The fetal mandible is discussed in detail by Wissmer ('27). The zygomatic bone is a flat membrane bone with identifiable infraorbital, frontal and zygomatic processes. As the malar tubercle is only slightly indicated the bone has a triangular outline instead of the adult diamond form. The bone has a uniform thin depth, for the orbital facies, which deepens the bone, is not well developed. A large unossified gap is present between the zygomatic processes of the zygomatic bone and the temporal bone. The palate bone has so differentiated that the horizontal and vertical lamina are approximately of equal size; and the orbital, sphenoidal and pyramidal processes and the incisura sphenopalatine are all recognizable. The two bilateral centers of the vomer are located on the inferior border of the medial nasal cartilages.

The open reticulated parietal bones show evidence of ossifying from one center. The interparietal bone is a line of open reticulated bone just superior to the supraoccipital bone. The two bones are connected medially by a sliver of bone. Although Augier ('31b) and La Coste ('31) stated that the interparietal bone fuses with the supraoccipital bone initially at the lateral margins, the specimens of this stage (fig. 2) and stage II (fig. 3) show that at least there may be an initial medial fusion between the two bones. The exoccipital bones, which lie inferior to the posterior border of the parietal bone at the level of the angle of the mandible, are in series with the more medially placed neural arch centers. The tympanic annulus is an ovoid nodule of bone located just inferior to the posterior border of the squamosal bone in the region of the future superior anterior region of the annulus. The squamosal bone with the slightly differentiated squama is mainly zygomatic process and squamal base. The mandibular fossa is undeveloped.

The clavicle is sigmoid shaped. The lateral edge is superolateral to the acromial process of the scapula and the medial edge is medial to the upper thoracic neural arches. The scapula is lunate. The curved vertebral border and the curved glenoid border, which is relatively large, are parallel to each other. The scapular spine is only slightly developed. There is no osseous acromial process. The ribs have almost reached the same proportions to each other and to the costal cartilages as exist during postnatal life. The angle and the body of each rib are well formed but the neck, tubercle and capitulum of each rib are unossified. The proximal ends of the ribs are in a parasagittal line that is lateral to the parasagittal line of the neural arches. In other words, the proximo-medial ends of the ribs of each side are relatively far apart.

Asymmetry in the appearance of bilateral ossification centers is illustrated by the cervical and thoracic neural arches present. Thoracic neural arch I is the largest arch center present and therefore it was presumably the first to appear.

Stage II (fig. 3)

Length, 56 mm. CR, 75 mm. CH.
Age, 78 days (approximately).

Bones present that were absent in stage I —

Facial bones: lacrimal bone.

Basicranial bone: basioccipital bone.

Vertebral column bones: neural arches from cervical 1 to lumbar 3 inclusive are present; except for thoracic centrum 7, thoracic centra from thoracic 6 to lumbar 4 inclusive are present.

Upper extremity bones: metacarpal diaphyses 1 and 5; proximal phalangeal diaphyses 2, 3 and 4.

Lower extremity bones: metatarsal diaphyses 1, 4 and 5; terminal phalangeal diaphyses 2, 3, 4 and 5.

Description —

The facial bones have increased in absolute size and, with respect to the facial area, in relative area; and they have reached a size that is almost proportional to their definitive area. The membranous calvarial bones, which have not as yet reached the size that is proportional to their definitive area, have grown rapidly in the interval since the last stage. The small alisphenoid bones, the elongated exoccipital bones and the nodular basiocciptal bone are the only osseous elements in the predominately cartilaginous basicranium.

The frontal processes of the maxillae have grown superiorly to flank the elongating nasal bones. The nasal notches are enlarged and the sutura notha is absent. The growth of the malar tubercle gives the corpus of the zygomatic bone a diamond shape. The thin attenuated maxillary process of the zygomatic bone extends to the region of the future infraorbital canal; and the orbital facies, which is commencing to form, gives the bone some depth. The vomer consists of the two lateral alae and the median ventral keel. Each parietal bone and each half of the interparietal bone shows evidence of ossifying from two centers of ossification, one superior to the other.

The zygomatic process of the squamosal bone has elongated almost to articulate with the zygomatic process of the zygomatic bone. The squama of the squamosal bone has three interconnected plates of bone radiating superiorly from the base of the bone. The tympanic annulus is a semicircular rod with an enlarged antero-superior portion.

The relatively more rapid growth of the vertebral border as compared with the glenoid border, since stage I (fig. 2) has altered the shape of the scapula. The scapular spine is larger than in stage I (fig. 2). The neural arches are small bars consisting of the radices and the laminae. They are further apart in the cervical region than in the thoracic and lumbar regions. The centra are larger than the arches. The great antero-posterior distance between the line of the neural arches and the line of the centra with respect to the depth of the thorax is marked.

Stage III (fig. 4)

Length, 88 mm. CR, 125 mm. CH.
Age, 3.25 lunar months (approximately).

Bones present that were absent in previous stages —

Basicranial bones: basisphenoid bone, orbitosphenoid bone.

Vertebral column bones: lumbar neural arches 4 and 5, and sacral neural arch 1, cervical centra 3, 4, and 5, lumbar centrum 5, and sacral centra 1, 2 and 3.

Upper extremity bones: all phalangeal diaphyseal centers are present.

Lower extremity bones: diaphyses of proximal phalanges 1, 2 and 3.

Description —

The facial bones have differentiated to a size that is proportional with respect to their definitive areas. The orbital facies of the zygomatic bone completes the general form of this bone. In the specimen used to illustrate this stage the metopic fontanelle appears to be formed as a result of the accelerated growth of the supero-medial border of the frontal squama of the frontal bone. The unossified notch on the posterior border of the parietal bone is probably an indication of a space separating the two parietal ossification centers. The tympanic annulus forms three quarters of a circle. The basisphenoid centers are paired nodules in the cartilaginous sella turcica and the orbitosphenoid bone is a nodule in the metopic root of the orbital cartilage.

By this stage the relative proportions of each osseous rib to its costal cartilage and to the other ribs are, in general, similar to these proportions in the adult.

The differentiation as well as the appearance of the neural arches follows a cranio-caudal pattern. The cervical arches have narrow laminae and radices while the lumbar neural arches are small osseous nodules. The laminae, where developed, are directed caudo-posteriorly from the radices with which they form approximately a 120 degree angle. The relative sizes of the centra are directly related to their order of appearance.

The alar portion of the ilium is formed, but is only slightly indicated. The angle of the greater sciatic notch is more obtuse than in later stages.

Stage IV (fig. 5)

Length, 105 mm. CR, 156 mm. CH. Age, 3.5 lunar months.

Bones present that were absent in previous stages —

Basicranial bones: lingula.

Pelvic bones: ischium.

Lower extremity bones: proximal phalangeal diaphyses 4 and 5, all middle phalangeal diaphyses.

Description —

In the specimen used the membranous area between the frontal squama of the frontal bone is large for this stage. A cartilaginous Kerkring's ossicle is present in the inferior supraoccipital notch. The basisphenoid has the shape of a block C with the vertical line of the C posterior in position. The lingula is a short rod extending laterally from the basisphenoid bone.

The laminae and radices of the neural arches are longer and broader than in previous stages. The nodular ischiatic bone is located in the cartilaginous ischiatic superior ramus.

Stage V (fig. 6)

Length, 139 mm. CR, 205 mm. CH. Age, 4.25 lunar months.

Bones present that were absent in previous stages —

Facial bones: maxillary turbinates.

Basicranial bones: posterior lateral presphenoid bones, incus, malleus, petrosal centers, ossicles of Bertini.

Thoracic bones: manubrium, right 7th cervical rib (inconstant).

Vertebral column bones: sacral neural arches 2 and 3, sacral centrum 1.

Lower extremity bones: middle phalangeal diaphyses 2 and 3.

Description —

At approximately this stage the membranous calvarial bones have differentiated to a size that is proportional with respect to their definitive areas (Inman, '33; Noback, '43). The sutural spaces of the calvarium are narrow and the fontanelles are discrete structures. The orbitosphenoid bone, shaped like a boomerang, is ossified in the regions of the anterior clinoid process and of the superior portion of the optic foramen. The alae of this bone are unossified. The paired postero-lateral presphenoid centers are small nodules in the lateral ends of the groove of the optic chiasma. The basisphenoid bone has invaded the dorsum sellae posteriorly and the tuberculum sella anteriorly. It is notched anteriorly. The incisura between the body and the squama of the alisphenoid bone representing the site of the future foramina ovale and the foramen spinosum is deep.

A supero-medial notch and rounded infero-lateral edges characterize many of the centra.

Stage VI (fig. 7)

Length, 175 mm. CR, 240 mm. CH. Age, 5 lunar months.

Bones present that were absent in previous stages —

Facial bones: superior concha.

Basicranial bones: anterior lateral presphenoid centers, stapes, petrosal centers, ethmoidal centers, ossicles of Bertini.

Vertebral column bones: dens (omitted in figures 7 and 8), neural arches 4 and 5.

Pelvic bones: pubis.

Lower extremity bones: calcaneus (all phalangeal diaphyses are present soon after this stage).

Description —

The cartilage bones of the skull are the most actively differentiating bones of the skull since the last stage as attested by the appearance of several cartilage ossification centers and by the relatively rapid growth of these bones. None of these cartilage bones have assumed a size that is proportional with respect to its definitive area. The small ethmoidal turbinate centers may be present in the superior and middle nasal conchae. No further ethmoidal ossification is indicated. The anterior lateral presphenoid centers may be present. The posterior presphenoid centers are elongated, extending toward the anterior ramus of the lesser wing of the sphenoid. The orbitosphenoid consists of a posterior ramus (anterior clinoid process), of an anterior ramus (supraoptic portion) and of a small lateral ala. Each lingula is broad. The ossification of the petrosum during this portion of fetal life is discussed by Bast ('30). In the lateral view the petrosum is visible posterior to the squamosal bone. This is due to the lateral growth of the petrosum subsequent to the appearance of its ossification centers. The large unossified petrosal foramen (fig. 7c) is the subarcuate fossa.

The form and anatomical relations of the post cranial bones are obvious in the figures.

Stage VII (fig. 8)

Length, 352 mm. CR, 520 mm. CH. Age, 10 lunar months.

Bones present that were absent in previous stages —

Facial bones: mental ossicles.

Basicranial bones: tympanohyal center.

Hyoid bones: body of the hyoid bone.

Thoracic bones: manubrium and first three sternal corpus centers (Borovanský, 1931).

Vertebral column bones: sacral centrum 5, sacral costal centers 1, 2 and 3 (omitted in figure 8).

Lower extremity bones: talus, distal femoral epiphysis, proximal tibial epiphysis.

Description —

The description of the newborn infant osseous skeleton is summarized by Hess ('17), Scammon ('23), Testut ('21), standard anatomical textbooks, and the literature.

Except for the ribs the cartilage bones have not as yet reached sizes that are proportional with respect to their definite areas. Broad cartilaginous synchondroses separate the bones from each other. The peripheral margins of the calvarial bones are characterized by short peripherally projecting osseous trabeculae while the calvarial suture spaces have a varying number of small osseous nodules of bones detached from the bone proper. These may possibly be potential sutural bones. The peripherally projecting osseous trabeculae of the margins of the bones are not characteristic of cartilage bones and they are not prominent features of the facial bones. The bone superior to the occipital bone is either an enlarged sutural bone or a preinterparietal bone (Augier, '31a).

The pubis and the ischium are restricted to the superior rami of their respective cartilages. Occasionally the epiphyses of the head of the humerus, the cuboid, the hamate, the cuneiform III bones and the epiphyses of the head of the femur are present in the newborn infant (see Noback, '43, for bibliography).

THE DEVELOPMENTAL ANATOMY OF SOME OF THE PRENATAL BONES

Some miscellaneous observations of the developmental anatomy of certain bones during prenatal life are presented. No attempt is made to give a complete account of the prenatal developmental anatomy of any bone, nor a complete discussion of the controversial aspects of any phase of the development of any bone.

Mandible. From an examination of the published and original roentgenograms and from cleared and stained embryos, fetuses and newborn infants, the prenatal and circumnatal mandible appears to be normally always depressed and not opposed to the maxillary bone as it is generally illustrated (figs. 2–8).

Frontal bone. The prenatal developmental anatomy of the frontal bone as presented by Inman and Saunders ('37) has been fully confirmed. Each half of the frontal bone ossifies from a single center appearing just above the superciliary ridge.

Parietal bone. The parietal bone ossifies from one ossification center (Rambaud and Renault, 1864; Pendergrass and Pepper, '39; Arey, '42) or from two ossification centers, appearing close together one above the other (Toldt, 1883; Augier, '31a). The normal ossification of the parietal bone from three or four ossification centers (reviewed by Bardeen, '10) is not acceptable as this conclusion is based on rare anomalies and not on any direct observations of the ossifying bone. The parietal bone of a series of nine cleared and stained fetuses ranging from 38 mm. CR to 71 mm. CR length showed evidence of ossifying from one center in one fetus of 38 mm. CR (fig. 2) and from two centers in eight fetuses (fig. 3 and fig. 4). All the parietal bones interpreted as ossifying from two centers, one superior to the other, have the constriction of the hour glass shape described by Mall ('06) on the anterior and posterior borders midway between the superior and inferior borders of the bone (fig. 3). The assumption is made that these constrictions are the remnants of the membranous spaces between two centers. On the basis of these observations it may be provisionally concluded that each parietal bone normally ossifies from two ossification centers.

Tympanic annulus. The tympanic annulus appears as a small round nodule, which represents the anterior superior portion of the annulus, just inferior to the posterior border of the temporal squama (fig. 2). With the posterior growth of the temporal squama the tympanic annulus becomes located just inferior to the junction of the zygomatic process of the temporal squama and the temporal squama proper. This relation is maintained throughout prenatal life. The annulus has a semicircular shape at the 56-mm. CR stage (fig. 3) and a three-quarter circle form at about the 75-mm. CR stage (fig. 4). During early fetal life the annulus has a small osseous appendage on its superior anterior border (fig. 2). The bone grows in depth until birth when it commences to fuse to the squamosal bone.

Occipital bone. The changes in the relative sizes of the interparietal portion (membrane bone) and the supraoccipital portion (cartilage bone) of the occipital bone illustrates the rapid growth of membrane bone as compared to slow growth of cartilage bone. Until the 88-mm. CR stage (fig. 4) the supraoccipital bone has a larger surface area than the interparietal bone. At approximately the 88-mm. CR stage these two bones have approximately the same surface area. After this stage the interparietal bone has a larger surface area than the supraoccpital bone. The interpretation of the ossification of the interparietal bone from one or two pairs of bilateral centers is discussed by Augier ('31b). No evidence was obtained to substantiate Mall's ('06) observation that the supraoccipital bone ossifies from four centers. It appears that this bone ossifies normally from one center.

Scapula. The changes in the form of the scapula during prenatal life may be expressed in terms of the relative growth of the axillary and vertebral borders. Throughout prenatal life the vertebral border is growing absolutely faster than the axillary border (figs. 2–8).

Clavicle. By stage I (fig. 2) the definition of the clavicle is similar to that of the mature clavicle. Unlike the postnatal clavicle, which lies in a horizontal plane, the fetal clavicle is inclined in an oblique plane from latero-superior to medio-inferior. The prenatal obliquity of the clavicle is present in all the cleared specimens examined and it is confirmed by published roentgenograms in the literature.

Ribs. At the 56-mm. CR stage (fig. 3) the breadth of each rib is the same throughout its length (the superior and inferior borders are almost parallel to each other). At the costal angle there are indications of a constriction in the depth of the ribs. Just distal to this constriction an increased breadth of the rib is definitely present by the 88-mm. CR stage (fig. 4). This increased depth, characteristic of the posterior 7 or 8 ribs, is due mainly to ossification on the inferior border of the ribs.

Vertebral column. Roentgenologists and anatomists refer to the cranio-caudal appearance of the primary neural arch ossification centers (Ruckensteiner, '31). However, except for an observation by Mall ('06), all published data report the findings of either no neural arch centers present in a specimen or never less than the cranial 9 neural arch centers. As the arch centers caudal to the second thoracic arch can be easily demonstrated to appear craniocaudally, the assumption has been made that the cranial 9 neural arches likewise appear craniocaudally. Mall ('06) described a 33-mm. CR stage fetus as having cervical arches 1, 2 and 7, and thoracic arches 1 and 2 present on the right side; and cervical arches 1, 2 and 3, and thoracic arches 1, 2, 3 and 4 on the left side. A fetus of the 38-mm. CR stage (fig. 2) has the cervical arches 3 and 5 and the thoracic arches 1 and 2 present on the right side; and cervical arches 3, 4, 5, 6 and 7, and thoracic arches 1 and 2 present on the left side. None of the other arches are present in either specimen. In both specimens the left side has the greater number of centers and the first thoracic arch center is the largest neural center. On the basis of these two specimens it may be suggested that the cervical neural arches do not appear craniocaudally, that the thoracic arch 1 is the first to appear, and that there is probably no systematic order of appearance of the cranial 9 arch centers. More data are needed to clarify the order of appearance of these centers.

On the basis of anomalous centra, several. authors (Beadle, '31, and others) have concluded that each centrum ossifies from two bilateral ossification centers. Most workers (Alexander, '06; Mall, '06 and others) are of the opinion that each centrum ossifies from one center. In the entire series of cleared and stained specimens examined in this study, all of the centra appeared to ossify from one center. The further development of the supero-medial notch on some of the centra, as noted in stage III (fig. 4), may explain the formation of the anomalous bipartite centra occasionally observed in postnatal life by Beadle ('31) and others.

Ilium. At the 38-mm. CR stage (fig. 2) the osseous ilium is enlarged anteriorly and attenuated posteriorly. The greater sciatic notch is large and its angle between the attenuated posterior portion of the ala and the cartilaginous iliac body is obtuse. By the 88-mm. CR stage (fig. 4) this angle of the greater sciatic notch is as acute as it is in the newborn infant. At the 175-mm. CR stage (fig. 7) the iliac crest, the anterior and posterior iliac spines, the greater sciatic notch and the ala proper resemble, in general, their definitive form. The body has not as yet fully invaded the acetabulum and a broad cartilaginous synchrondrosis separates it from the ischium and the pubis. The relative distance between the twelfth rib and the superior border of the ilium, and the cranio-caudal distance between the fifth lumbar vertebra and the superior border of the ilium are reduced with fetal age (fig. 2–8). The secondary iliac ossification centers appearing during prenatal life in the region of the posterior inferior iliac spine and the anterior iliac spine, described by Lambertz ('00) but not seen by Adair ('18), were not observed.

CENTRIC AND ECCENTRIC OSSIFICATION CENTERS

"Every bone develops from one or more bony nuclei or centers of ossification. The localization of this center is subject to but slight variation" (Spalteholz, '33). On the basis of the relation of the location of an ossification center to the geometric center of the bone or of the part of the bone that the center forms, there are two types of ossification centers, (1) centric centers and (2) eccentric centers. A centric center is an ossification center which appears approximately in the geometric center of the bone or of the part of the bone that the center forms. An eccentric center is an ossification center which appears in a loci removed from the geometric center of the bone or of the part of the bone that the center forms.

The prenatally ossifying bones with centric centers are the zygomatic bone, lacrimal bone, nasal bone, palate bone, vertebral centra, clavicle, diaphyses of the long bones of the extremities, body of the hyoid bone, basioccipital bone, cuboid bone and the talus. The palate center appears at the junction of the horizontal and vertical plates (Mall, '06) or in the vertical plate immediately internal to the palatine nerves (Fawcett, '10). The basisphenoid bone and the presphenoid bone are both ossified from more than one ossification center. The fusion of these centers occurs soon after their appearance and thus these two bones may be considered as ossifying from one multiple center. If this premise is accepted these bones may be considered as ossifying from centric centers; otherwise, from eccentric centers. The locations of the other centric centers require no explanation.

The prenatally ossifying bones with eccentric centers are the frontal bone, parietal bone, mandible, maxillary bone, squamosal bone, tympanic annulus, interparietal bone, supraoccipital bone, orbitosphenoid bone, alisphenoid bone, vomer, lingula, medial pterygoid plate, exoccipital bone, inferior concha, petrosum, scapula, ribs, ilium, ischium, pubis and calcaneus.

The frontal bone's center appears just superior to the superciliary ridge (Inman and Saunders, '37), while the geometric center of the definitive bone is higher on the frontal squama. The parietal bone ossifies from two centers, one superior to the other (see above). The mandibular center appears at a point one-third of the length of the bone from the symphysis (Fawcett, '05; Low, '09). The maxillary center ossifies on the outer side of the nasal cavity just above the cuspid tooth germ (Mall, '06). The center for the squamosal portion of the temporal bone arises in the region of the midpoint of the base of the squama (fig. 2). The tympanic annulus appears at the antero-superior region of the bone (fig. 2). The bilateral centers of the interparietal bone appear at the inferior border of the bone just lateral to the midsagittal line from which region ossification proceeds superiorly, laterally and medially (LaCoste, '31; Augier, '31b) (fig. 2). The center of the orbitosphenoid bone which appears in the metopic root of the orbital cartilage (Mall, '06), and the center of the alisphenoid bone which appears lateral and inferior to the foramen rotundum (Sutton, 1885) are located in the medial portions of their respective bones. Subsequent ossification is predominately lateral in each bone while in the alisphenoid bone ossification also extends inferiorly into the lateral pterygoid plate. The medial pterygoid plate center appears in the region of the hamulus and ossification extends superiorly (Fawcett, '10). The center of the lingula appears basally (Rambaud and Renault, 1864) and the main ossification extends distally. The vomerine centers appear as bilateral centers in the inferior portion of the definitive bone and subsequent ossification proceeds predominately superiorly into the alae (Fawcett, '10). The exoccipital center appears in the region of the hypoglossal foramen while the inferior concha appears in the supero-medial aspect of the inferior chonchal cartilage. The multiple centers of the petrosal portion of the temporal bone appear adjacent to the membranous labyrinth (Bast, '30).

The scapula center ossifies in the corpus in the middle of the spine (Mall, '06) while the neural arch centers appear at the base of the superior articular processes in the laminae (Rambaud and Renault, 1864). The rib centers ossify at the angle of the costal cartilages. The iliac center arises in the inferior medial portion of the ala above the greater sciatic notch (Lambertz, '00; Falk, '01) while the ischiatic and pubic centers appear in their respective superior rami (Rambaud and Renault, 1864). The calcaneal center arises slightly anterior to the center of the calcaneal cartilage (Bryce, '15).

The sites of the frontal eminence and the parietal eminence are not necessarily coincident with the sites of the ossification centers of these bones. The eminences are evident at the region of the greatest curvature and thus they seem to be a response to a mechanical stimulus. The ossification centers appear in a constant location, a condition which would seem to indicate a response to a genetic stimulus. The frontal center appears below the site of the future frontal eminence. In a parietal bone which arises from one center, the locations of the eminence and the center of ossification could be coincident. When the early fetal parietal bone, ossifying from two centers, is enlarged to the definitive size, the site of the future parietal eminence can be plotted as being located between the two centers. Thus the absolute coincidence of the location of the eminences and the ossification centers of the frontal bone and the parietal bone does not appear to be acceptable.

ASYMMETRY IN THE TIME OF APPEARANCE OF THE BILATERAL OSSIFICATION CENTERS

The unilateral presence of one and the unilateral absence of the other of a pair of bilateral ossification centers during prenatal and circumnatal life has been reported for the lacrimal bone in a 4.25-cm. fetus by Macalister (1884); for some ribs in a 30-mm. CR embryo, for some cervical and upper thoracic neural arches in a 34-mm. CR fetus (noted above) and of the tympanic annulus of a 42-mm. CR embryo by Mall ('06); for some of the sternal centers by Borovanský ('31); and of thirty-two cases of seven different circumnatally appearing centers (head of the humerus, coracoid process, capitate, hamate, distal femoral epiphysis, cuboid and external cuneiform) in 640 newborn infants by Menees and Holly ('32). Borovanský and Hnévkovský ('29), Pryor ('36) and Flecker ('42) present similar data for some of the bones appearing after birth.

The asymmetrical presence of some of the cervical and upper neural arches is discussed above and illustrated in stage I (fig. 2). The posterior lateral presphenoid center was present on the left side and absent on the right side in the fetus illustrated by stage III (fig. 4). The twelfth rib was present on the left side and absent on the right side of a 56-mm. CR fetus, and the right twelfth rib was much larger than the left twelfth rib in the fetus illustrated by stage I (fig. 2). In the latter case the assumption is made that the larger rib appeared first.

The paucity of reported observations of the asymmetrical appearance of ossification centers during early fetal life, when most centers are appearing, may possibly be accounted for by the infrequent use of sensitive techniques, like the clearing and staining methods, in examining specimens; and by the fact that bilateral centers probably appear at the same time or within a short time of each other. In some cases the interpretation of the congenital absence of a bone is possible.

These observations suggest that although the asymmetrical appearance of bilateral centers occasionally occurs the appearance of bilateral centers is essentially symmetrical.

THE "SEQUENCE" OF CRANIO-CAUDAL DEVELOPMENT AND THE APPEARANCE OF THE OSSIFICATION CENTERS

The sequence of the appearance of the ossification centers during prenatal life shows varying degrees of conformity and nonconformity to the "sequence" of cranio-caudal development (the so-called "law" of antero-posterior development or "law" of developmental direction).

The regional order of the appearance of the primary ossification centers during prenatal life appears to be roughly as follows: (1) Diaphyseal centers of the two proximal segments of the extremities, (2) centers of the facial bones, (3) and (4) centers of the calvarial bones and centers of the thoracic bones (except for the centers of the sternum), (5) primary centers of the free vertebral column, (6) diaphyseal centers of the hand, (7) and (8) centers of the basicranial bones (except for the ethmoidal centers) and centers of the pelvic bones, (9) diaphyseal centers of the foot, (10) centers of the sternum, and (11) centers of the hyoid complex (Mall, '06; Adair, '18; Augier, '31a; Ruckensteiner, '31; Noback, '43, and others). From this order it may be concluded that the sequence of the time of the appearance of the ossification centers of the body as a whole does not fully conform to the "sequence" of cranio-caudal development.

The intraregional order of appearance of some groups of ossification centers does conform with the "sequence" of cranio-caudal development. For example, the order of appearance of the head branchial arch centers is (1) centers of the facial bones, (2) centers of the calvarial bones, (3) centers of the basicranial bones and (4) centers of the hyoid bones. The centers of the sternum appear cranio-caudally (Borovanský, '31). Except for the first phalangeal centers, the diaphyseal centers of each row of the metacarpals the metatarsals and the phalanges appear, as a rule, cranio-caudally with respect to their embryonic position (Mall, '06, Ruckensteiner, '31). With possible exception of the cervical neural arches, the neural arch centers appear cranio-caudally (Mall, '06).

The intraregional order of appearance of other groups of ossification centers show degrees from partial to total non-conformity to the "sequence". The order of appearance of the vertebral centra, the ribs and the diaphyses of the upper and lower extremities are examples. The lower thoracic are the first centra centers to appear. The sixth and seventh costal centers are probably the first ribs to appear. Subsequent ossification in these two groups of bones is cephalad and caudad to the initially appearing centers (Mall, '06). Those centers that appear caudally conform to the "sequence" while those appearing cranially do not. The diaphyseal centers of the upper extremity ossify in the following sequence: (a) humerus, (b) radius, (c) ulna, (d) metacarpals, (e) terminal phalanges, (f) basal phalanges, (g) middle phalanges, and (h) carpals. The diaphyseal centers of the lower extremities ossify in the same order but later than those of the upper extremity. The appearance of the more proximal elements follows the ''sequence'' while the appearance of the more distal elements does not appear to do so.

SHORT CRITIQUE OF THE LITERATURE OF THE TIME OF THE APEARANCE OF THE OSSIFICATION CENTERS

Pathology, race, sex, nutrition and other factors have been shown to influence the time of the appearance of ossification. To obtain sufficient data to properly evaluate these factors, consolidation of the data in the literature should be helpful. However, even a short review of the literature reveals the difficulty of such consolidation because of the omission of much vital data and because of the variety of techniques employed in observing the prenatal skeleton.

The ages of the specimens examined are often given but the method used to calculate fetal age or the linear dimensions of the specimens are frequently omitted. The number of specimens examined is often omitted. In order to get an accurate idea concerning the range of the time of the appearance of ossification centers the absence of a center should be reported. Most papers only report the earliest observed presence of an ossification center. Many recent publications ignore the data of accurate investigations (Flecker, '42).

To obtain an accurate consolidation of data, account must be taken of the techniques employed in observing the time of the appearance of the centers. Ossification of a given center is observed first by the sectioning technique, followed in order by the KOH clearing and alizarin red S staining technique, by the roentgenographic method and by gross dissection. The time difference between the clearing and staining technique and the roentgenographic technique, in indicating the appearance of a center is approximately 1 week (Hess, '17). A striking difference illustrating the relative sensitivity of these two techniques in revealing calcification is shown by the parietal bone which, according to Mall ('06) using the clearing technique, appears at the 31 mm. CR stage and which, according to Hill ('39) using the roentgenographic method, appears at the 194-mm. CR stage.

SUMMARY

1. Aspects of the developmental anatomy of the prenatal and circumnatal macroscopic osseous skeleton are outlined and illustrated in seven stages. These stages are the 38-mm. CR, 56-mm. CR, 88-mm. CR, 105-mm. CR, 139-mm. CR, 175-mm. CR and 352-mm. CR stages.

2. Some special phases of the normal prenatal developmental anatomy of the mandible, frontal bone, parietal bone, tympanic annulus, occipital bone, scapula, clavicle, ribs, centra, neural arches and ilium are briefly discussed.

3. On the basis of the relation of the location of an ossification center to the geometric center of the bone or the part of the bone that the center forms, there are two types of ossification centers, viz., (1) centric centers and (2) eccentric centers. A centric center is an ossification center which appears approximately in the geometric center of the bone or the part of the bone that the center forms. An eccentric center is an ossification center which appears in a loci removed from the geometric center of the bone or the part of the bone that the center forms. Each prenatal ossification center is classified into one of these two types.

4. The asymmetrical time of appearance of the bilateral ossification centers and the relation of the order of appearance of the ossification centers appearing during the prenatal and circumnatal periods to the ''sequence'' of cranio-caudal development are briefly discussed. Although the asymmetrical appearance of the bilateral centers occasionally occurs their appearance is essentially symmetrical. The sequence of the appearance of the ossification centers during prenatal life

shows varying degrees of conformity and nonconformity to the "sequence" of cranio-caudal development.

5. A short critique of the literature of the time of the appearance of the prenatal ossification centers is presented.

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EXPLANATION OF PLATES 1 TO 4

Each plate consists of two figures. Each figure (except fig. 1) illustrates three views, anterior, posterior, and lateral of one of the seven developmental stages discussed in the observations. Figure 1 is a key figure to facilitate the interpretation of figures 2 to 8 inclusive. All figures are drawn to the same crown-rump length. The manner of drawing the figures is outlined in the material and methods.

The increased flexibility of the vertebral column in the fragile cleared specimens is reflected in the figures by the degree of the column's curvature. When the curvature of the thoracic segment of the vertebral column is reduced there is a reduction in the obliquity of the ribs. Conversely, when there is an increase in the curvature of the thoracic segment of the vertebral column, there is an increase in the obliquity of the ribs. The anterior view of the specimen in figure 4 illustrates the horizontal position of the ribs when the vertebral column is held straight. Varying degrees of costal obliquity are illustrated in the other figures.

ABBREVIATIONS

Ali., alisphenoid bone (great wing of sphenoid bone)
Bo., basioccipital bone
Eo., exoccipital bone
Ip., interparietal bone
Is., ischium
K.O., Kerkring's ossicle
P., palate bone
Pe., petrosum
Pu., pubis
So., supraoccipital bone
T.A., tympanic annulus (tympanic bone)
Z., zygomatic bone

PLATE 1

EXPLANATION OF FIGURES

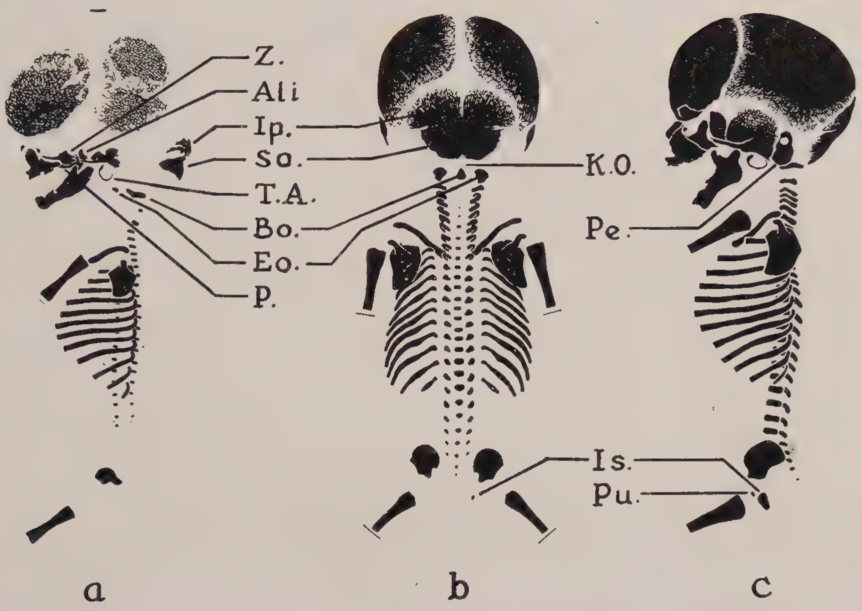
1 Three fetuses at different age stages with the bones, which are not easily recognizable, labeled to facilitate the interpretation of figures 2 to 8 inclusive.

- (a) Lateral view of fetus approximately 78 days old (56-mm. CR, 75-mm. CH).
- (b) Posterior view of a fetus approximately 3.5 lunar months old (105-mm. CR, 156-mm. CH).
- (c) Lateral view of a fetus approximately 5 lunar months old (175 mm. CR, 255-mm. CH).

2 Three drawings of the osseous skeleton of a human fetus approximately 64 days old (38-mm. CR) illustrating stage I.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

Figure 1


Z.
Ali
Ip.
So.
T.A.
Bo.
Eo.
P.
K.O.
Pe.
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Pu.
a
b
c

Figure 2

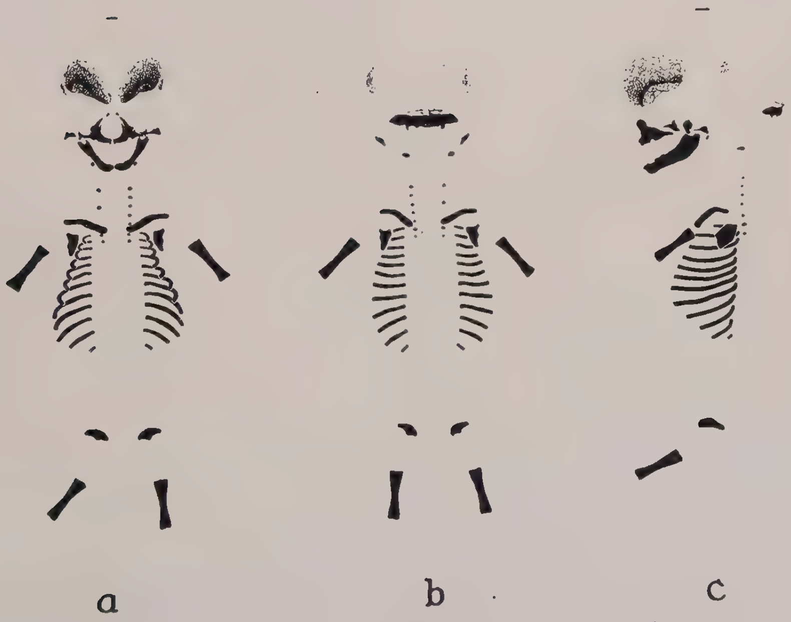

PLATE 2

EXPLANATION OF FIGURES

3 Three drawings of the osseous skeleton of a human fetus approximately 78 days old (56-mm. CR) illustrating stage II.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

4 Three drawings of the osseous skeleton of a human fetus approximately 3.25 lunar months (88-mm. CR) illustrating stage III.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

Figure 3

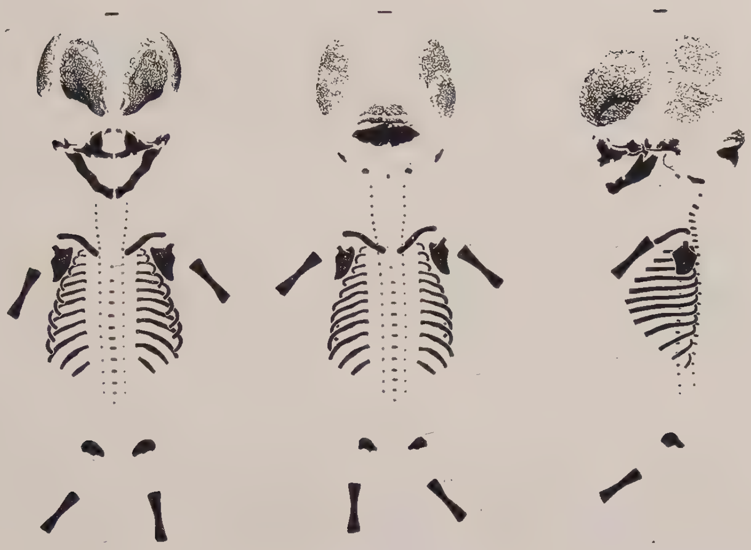

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Figure 4

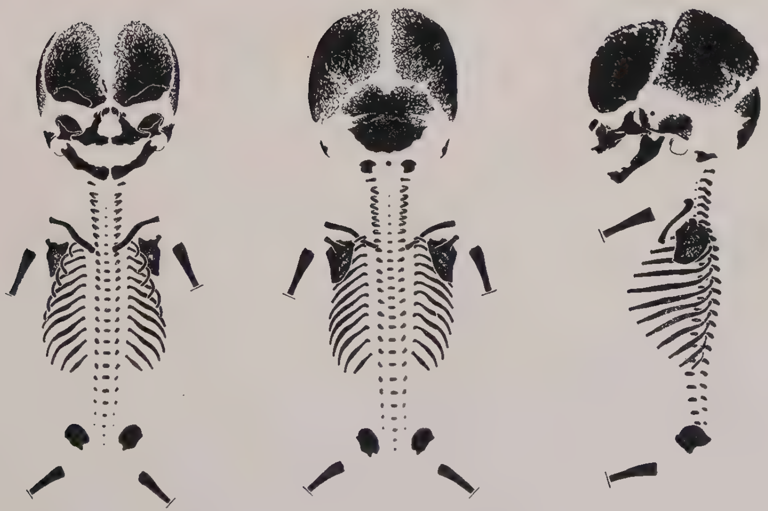

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PLATE 3

EXPLANATION OF FIGURES

5 Three drawings of the osseous skeleton of a human fetus 3.5 lunar months (105-mm. CR) illustrating stage IV.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

6 Three drawings of the osseous skeleton of a human fetus 4.25 lunar months (139-mm. CR) illustrating stage V.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

Figure 5

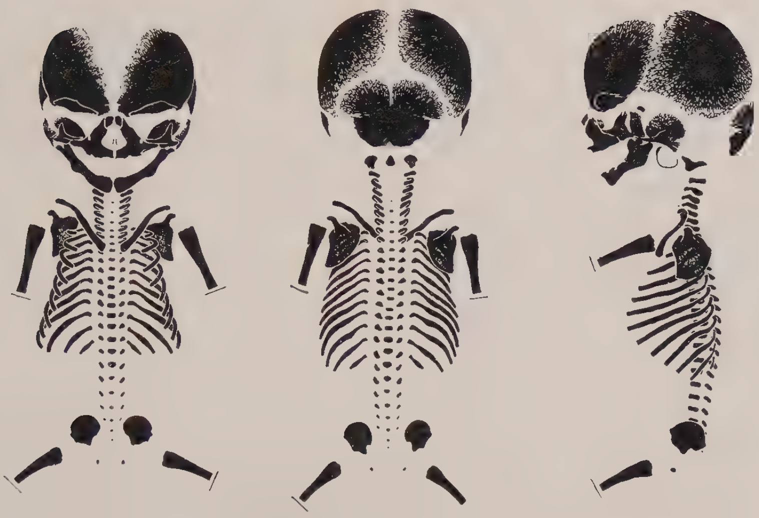

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Figure 6

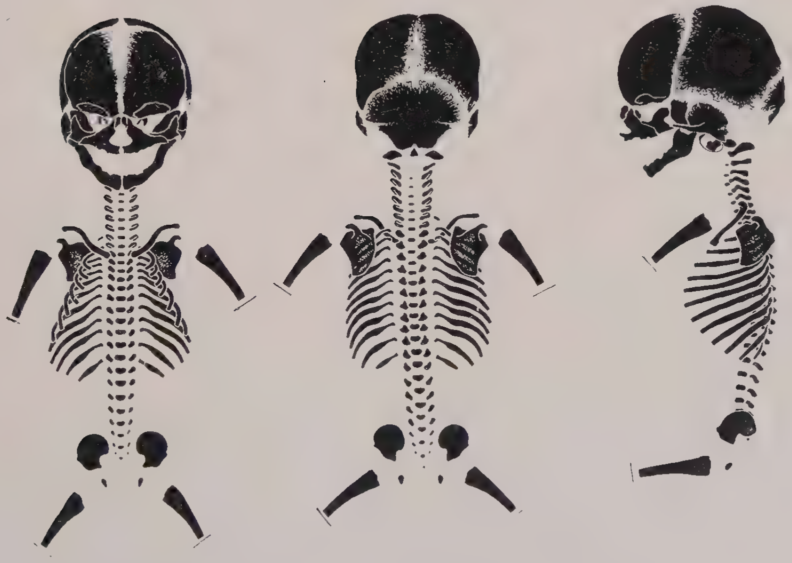

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PLATE 4

EXPLANATION OF FIGURES

7 Three drawings of the osseous skeleton of a human fetus 5 lunar months (175-mm. CR) illustrating stage VI.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

8 Three drawings of the osseous skeleton of a human fetus 10 lunar months (352-mm. CR) illustrating stage VII.

(a) Anterior view. (b) Posterior view. (c) Lateral view.

Figure 7

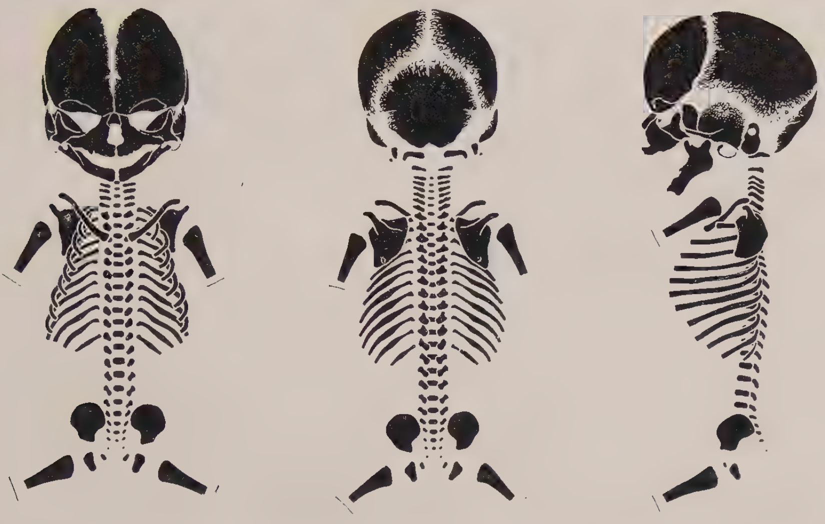

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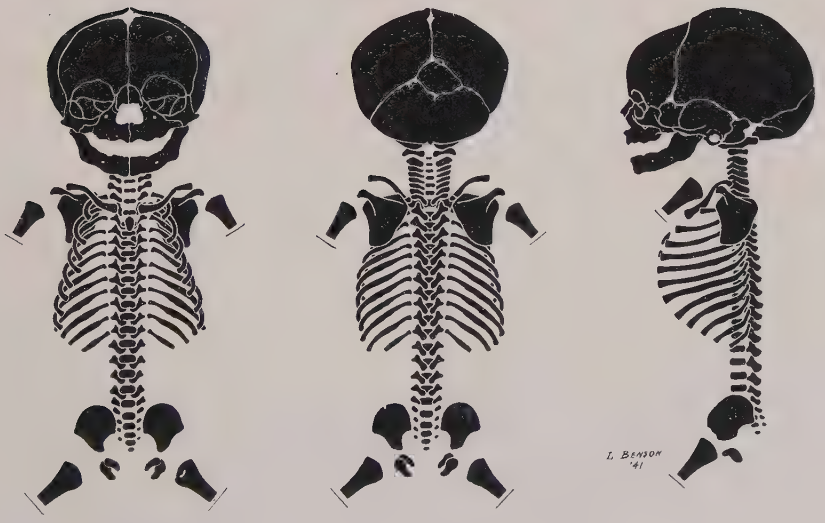
Figure 8



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VERTEBRAL LEVEL OF TERMINATION OF THE SPINAL CORD WITH REPORT OF A CASE OF SACRAL CORD[1]

ARTHUR F. REIMANN AND BARRY J. ANSON
Department of Anatomy. Northwestern University Medical School, Chicago, Illinois

SEVEN FIGURES

Prior to the year 1894, investigators paid scant attention to the wide variations in the level of termination of the spinal cord. Nuhn (1849), Pfitzner (1884) and Reid (1889) studied the lengths of individual segments of the spinal cord in a small number of specimens, for the purpose of demonstrating the extent of recession of the spinal cord during body-growth, and of affording a basis for determining the position of cord segments in relation to surface landmarks. Only occasional mention was made of departure from the accepted level of termination, namely, the first and second lumbar vertebrae.

Thompson and associates (1894), McCotter ('16) and Needles ('35) made the chief contributions to present-day knowledge of termination of the spinal cord. The site of vertebral termination, according to these investigators, varies between the middle of the twelfth thoracic and the lower third of the third lumbar; in approximately one-half of their cases the cord terminated in the vertebral thirds adjacent to the intervertebral disc between the first and second lumbar vertebrae. It was recognized that there is a tendency for the spinal cord in females to end at a lower level than in males. The point of termination in negroes was also found to be somewhat lower than in whites. No definite correlation was found to exist between height of body and length of vertebral column, or between either of these and the length of the spinal cord.

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[1] Contribution no. 376 from the Anatomical Laboratory of Northwestern University Medical School.

MATERIALS AND METHODS

The present report is based upon consecutive records of 129 adult specimens (105 male, 24 female, American whites and negroes).

The spinal cord was exposed by laminectomy and incision of the dural sac and arachnoid membrane. The point taken as the termination of the cord was that marking the junction of the medullary cone and the terminal filament, a point which coincides approximately with the emergence of the last spinal (coccygeal) nerve; there, according to the histological studies of Tarlov ('38); Harmeier ('33) and Needles, spinal gray substance becomes negligible in amount. The point was then established in relation to a third of a vertebra, or to an intervertebral disc.

In one specimen the spinal medulla extended to the level of the third sacral vertebra. In this cadaver, of an adult male negro, dissection was extended to include the lumbar and sacral plexuses and nerves, the muscles of the lower extremities, the bones of the pelvis, and lumbar part of the vertebral column.

The original data, converted into percentages of the total 129 specimens, are presented graphically in relation to a typical vertebral column. In order to demonstrate effectively the ordinary extent of retraction, an adult cord is illustrated in association with the cord of a 2½ month fetus.[2]

OBSERVATIONS AND DISCUSSION

In the current series, exclusive of the specimen of sacral cord, termination of the spinal cord ranges from the level of

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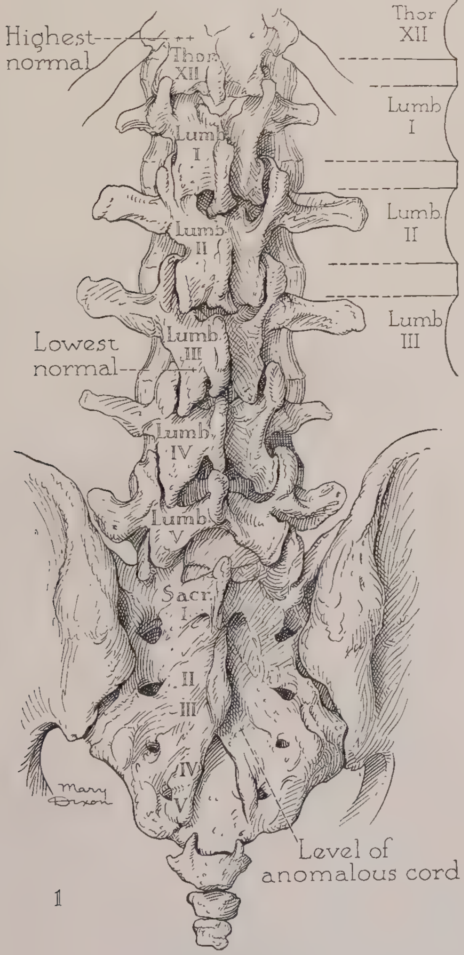
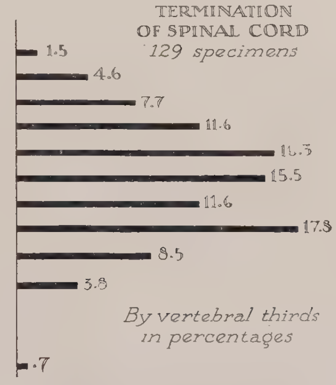
[2] The drawing for figure 3 was prepared at two and one-half times natural size, through the use of hand-lens, in order to record details of anatomical relationship which could not be recorded in an unenlarged drawing; all other drawings were natural size. In figure 5 the spinal structures are depicted as they were seen; the details of skeletal anatomy were added from examination of an articulated specimen which, prior to laminectomy, was used also for figure 1.

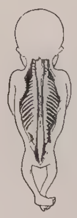
the lower third of the twelfth thoracic vertebra to that of the middle third of the third lumbar (fig. 1); the spread, then, is slightly less than that reported by Needles. Within the segments included between the lower third of the first and the


Highest normal
Thor XII
Lumb I
Lumb II
Lumb III
Lumb IV
Lumb V
Sacr. I
II
III
IV
V
Lowest normal
Thor XII
Lumb I
Lumb II
Lumb III
Level of anomalous cord
Mary Dixon

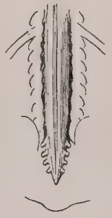
1



1a



2



3

Fig. 1 Posterior aspect of the lumbosacral vertebral column in an adult × ½.

Fig. 1a Graph, recording point of termination of the spinal cord in 128 specimens.

Fig. 2 Posterior aspect of the spinal cord, exposed by laminectomy, in a fetus of 2½ months × ½.

Fig. 3 Lumbosacral area of the same specimen × 1. The spinal cord occupies the entire length of the vertebral canal.

upper third of the second lumbar vertebrae, 43.4% of the total number are included, 68% within area of segments included between the middle thirds of the first and second lumbar vertebrae (fig. 1a). The level of the mean for the series falls at the disc between the first and second lumbar vertebrae (cf. fig. 6, all authors).

By combining the present set with others reported in the literature, a total of 801 records were brought into a common system. The scheme employed by Needles was adopted, in which each vertebra is divided into thirds, the neighboring half of the intervertebral disc being included with its adjacent upper or lower third of a vertebra. Whenever several cords terminated at a disc, that number was divided in half, each set being then allocated to its adjacent vertebral third (table 1). When the data were so arranged, it was found that within the confines of the lower third of the first lumbar segment and the upper third of the second lumbar vertebrae the spinal cord terminated in 43.4% of the current series, 49.6% of percent of Needles', 48.6% of McCotter's, 62.1% of Thompson's, or in 51.1% of the combined sets (fig. 6).

TABLE 1

Vertebral level of termination of the spinal cord.

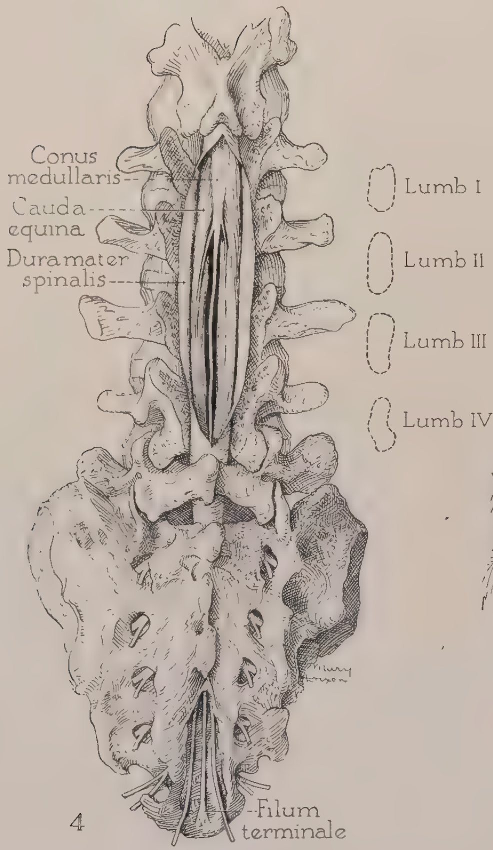
VERTEBRAL THIRDS		PRESENT SERIES		NEEDLES		MC COTTER		THOMPSON		TOTAL	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
T12	Middle	0	3.9	2	0.83	0		1	0.5	3	0.4
	Lower	5	3.9	12	5.0	3	0.9	2	1.01	22	2.7
	Upper	13	10.0	20	8.3	19	6.7	12	6.1	64	8.0
L1	Middle	15	11.6	17	7.1	35	15.2	26	13.1	93	11.6
	Lower	31	24.0	58	24.2	64	28.1	74	37.4	227	28.3
	Upper	25	19.4	61	25.4	48	20.5	49	24.7	183	22.8
L2	Middle	23	17.8	36	15.0	30	13.4	17	8.6	106	13.2
	Lower	14	10.8	26	10.4	31	13.9	16	8.1	87	10.8
	Upper	2	1.5	5	2.1	4	1.4	1	0.5	12	1.5
L3	Middle	1	0.8	1	0.4	0		0		2	0.2
	Lower	0		1	0.8	0		0		1	0.1
Totals		129		240		234		198		801	

Only Needles' series compares an adequate sample of both whites (107) and negroes (133). By arranging both groups into class intervals and by calculating the standard error of the difference between the means, it may be shown that fair agreement exists in the level of termination of spinal cords between whites and negroes.

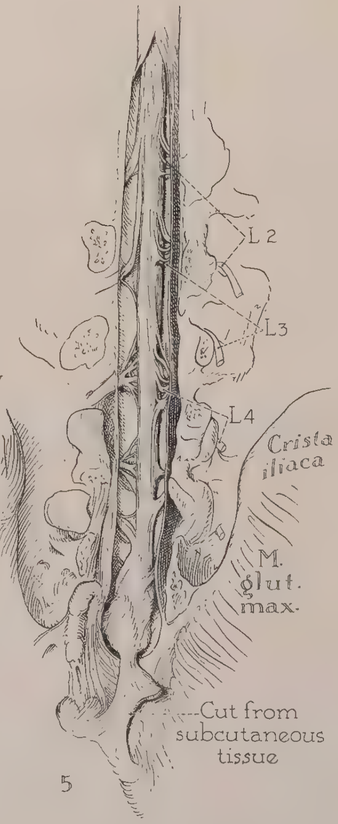
On the other hand, a comparison of sex differences in Thompson's series (115 males, 83 females) by the same method indicates a significant difference wherein the mean level of termination for the female group is a vertebral third lower than that for the opposite sex. The range for the female group is shorter than that for the male, the termination more variable.

As is well known, the fetal spinal cord undergoes recession within the vertebral canal. Cord and column are of equal length in a 3-month fetus (figs. 2 and 3), but thereafter differential growth is such that, the adult length of the vertebral column is approximately twenty-two times the fetal length, while the spinal cord has increased its length by but twelve times (fig. 4). In rare specimens, however, the cord is not drawn away from the point of its primordial sacral fixation; as a result, the spinal medulla occupies the lower lumbar and the sacral portions of the vertebral canal. In the present authors' series this type of variation is represented by a single case; it is, incidentally, the only case of sacral cord encountered in the course of 650 laminectomies. Because of the rarity of this condition, the case deserves special comment. In this specimen the calf of the right leg was atrophied, and the extremity was 3 cm. shorter than the left. There was no evidence of hypertrichosis, but a slight talipes equinovarus deformity was present in the right foot.

The skin over the midsacral area was slightly dimpled. Superficial dissection disclosed asymmetry of the sacrum with a depressed oval defect replacing the third, fourth and fifth sacral spines. A fibrous tag, issuing from the tough membranous cover of this osseous defect, connected membrane with superficial fascia over the lower end of the sacrum (fig. 5).


Conus medullaris
Cauda equina
Dura mater spinalis
Lumb I
Lumb II
Lumb III
Lumb IV
Filum terminale

4


L 2
L3
L4
Crista iliaca
M. glut. max.
Cut from subcutaneous tissue

5

Fig. 4 Posterior aspect of a normal spinal cord in an adult, exposed by laminectomy. The spinal cord proper ends opposite the first lumbar vertebra, while the terminal filament is prolonged to a coccygeal attachment × ½. To the right of figure 4 the positions of the spinous processes are recorded by horizontal projection; each is caudal to the level of the corresponding vertebral body.

Fig. 5 Posterior view of an anomalous spinal cord in an adult × ½. The cord, ending in a bulbous mass, reaches the level of the third sacral piece a distal tag (cut) passes through a hiatus at the usual site of the middle sacral crest, to become continuous with subcutaneous tissue.

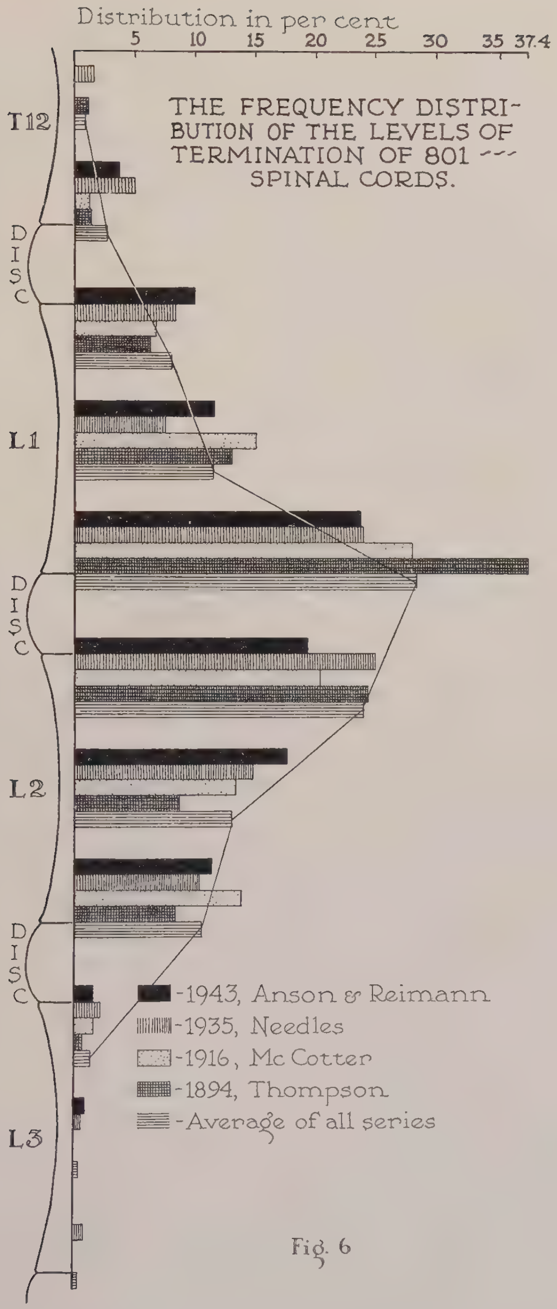


Fig. 6 Histogram based upon available large series, arranged in such a way that comparisons between sets of records may be made readily; within vertebral thirds the four sets of data are arranged in the same order.

The epidural space was obliterated inferior to the level of the first sacral segment. The dura mater and the arachnoid membrane were in contact with an enlarged sacral vertebral canal and fused caudally to the edges of the oval defect mentioned above. A large subarachnoid sac was thus formed in the sacral canal; it was 4 cm. in width and 2.5 cm. in depth at the level of the second sacral segment, and extended farther to the left than to the right side. The sacral canal ended blindly at the middle of the fourth sacral segment. The cleaned sacrum was found to be 8.5 cm. long, the right margin shorter than the left. The first of the three coccygeal pieces was fused to the fifth sacral segment. There was complete absence of bone in the regular location of the right halves of the second and third sacral bodies, while the left halves were wedge-shaped outline (fig. 7).

The spinal medulla extended caudalward to the second sacral level; it terminated in a small tumorous enlargement continuous with a fibrous tissue mass that was firmly attached to the membranous covering of the posterior bony defect in the sacrum. Spinal nerve roots below the first sacral were enmeshed in the fibrous tissue mass. The cord and its terminal enlargment were thus held close to the posterior wall of the canal; a capacious subarachnoid space lay anterior to these structures. The effects of low sacral fixation of the spinal medulla were evident in the lengthening and dislocation of thoracic and lumbar cord segments. Segments of the spinal medulla measured from 2.4 cm. at the tenth thoracic level to 5.4 cm. at the fourth lumbar level. Normally, no segment of the cord below the tenth thoracic is over 2.0 cm. long, the segments together from that to the fifth lumbar level being about 12.8 cm. long. The same linear group in the present specimen (T10 to L5) measured 27.0 cm. in length. The right side of the lumbar cord was locally lengthened more than the left; the lower part of the fourth lumbar roots, all of the fifth lumbar and first and second sacral roots ascended to their dural openings, obliquite increasing the lower the point of origin of the root (fig. 5).

Histologically, the distal enlargment consisted almost entirely of adipose tissue; at the periphery a few nerve-fibers were present, all of which coursed in a disorganized manner. The tissue contained an unusually large number of blood vessels.

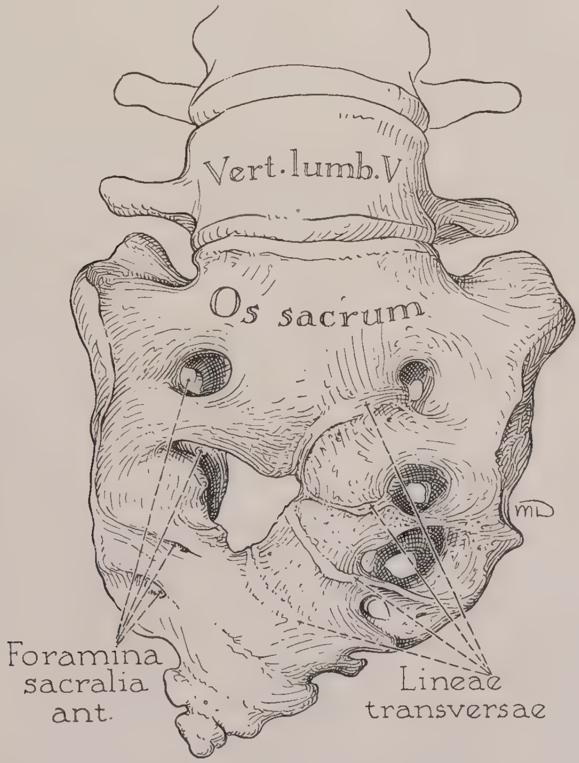


Fig. 7 Anterior aspect of the sacrum from the specimen with sacral cord × ½.

The left sacral plexus was normal in form; its constituent nerves and muscles in which they terminated were grossly normal, with the exception of the left piriformis muscle which was wholly replaced by fat. The right sacral plexus apparently contained only the branches of the upper four anterior sacral nerves; the left sciatic nerve measured 1.4 cm. in width, the right 1.0 cm. In the right lower extremity both simple atrophy and fatty degeneration had occured in the lower parts of the glutei maximus and medius, in all of the short external rotators, and in most of the crural muscles, but without selective difference between the groups of peroneal or tibial nerve innervation.

This anomaly conforms in major respects to the celebrated one described by von Recklinghausen (1886) and to those reviewed by Paul ('09) and Kermauner ('09). With the possible exception of the case of the first of these authors, the majority of the fully reported cases of sacral cord with associated spina bifida are, like that of Dittrich's ('30), in very young individuals in the immediate prenatal or postnatal age groups. The present specimen, however, is an adult. The occurrence of terminal tumor formation associated with low sacral fixation of the spinal cord has been described in several earlier reports, though the intradural position of such a terminal enlargement is not found in all cases.

The retention of the embryonic point of attachment of the cord in the adult may be reflected in certain far-reaching changes in the central nervous system. Russel and Donald ('35) stressed this as an important factor in the production of Arnold-Chiari deformities.[3] Under the term spinal dysraphism Lichtenstein ('42) recently pointed out that, where low fixation occurs, the cord must migrate caudally, as evidenced by the ascent of certain nerve roots, or the cord must lengthen. It is clear that in the present authors' case the latter process had taken place in the lumbar segments, as the measurements of cord segment lengths indicate. With such attenuation of the spinal cord, little downward traction would be expected in the region of the medulla oblongata.

The dilation of the dura-arachnoid sac and sacral canal was explained by von Recklinghausen and Paul as fetal meningocele which later became reduced in size for inclusion within the vertebral canal. More recently, Ingraham and Lowrey ('43) actually found an intraspinal, but extradural, meningocele associated with an occult lumbosacral spina bifida, thus demonstrating the existence of a condition which had been hypothesized by the earlier investigators.

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[3] The upper portion of the present authors' specimen had been dissected earlier; hence, no record can be furnished as to the form of the brain.

CONCLUSIONS AND SUMMARY

With the exception of a single specimen (sacral cord) the present authors' series of 129 adult specimens shows a range of variation in termination of the spinal cord from the lower third of the twelfth thoracic vertebra to the middle third of the third lumbar. The level of the mean is opposite the disc between the first and second lumbar vertebrae; 94% of the cords in the current series terminate within the territory of the upper two lumbar vertebrae.

A compilation of 672 similar records from the literature shows a range from the middle of the twelfth thoracic vertebra to the lower third of the third lumbar vertebra. The point of greatest frequency of termination for this more inclusive group is the lower third of the first lumbar segment; in the interval between the upper third of the second lumbar and the lower third of the first lumbar levels 51.1% of the group terminate.

A case of sacral cord, with anterior sacral defect and spina bifida occulta, is described and figured.

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BOOK REVIEW

AN ATLAS OF THE BASAL GANGLIA, BRAIN STEM AND SPINAL CORD. BASED ON MYELIN-STAINED MATERIAL. By H. A. Riley, professor of neurology and neuroanatomy, Columbia University, N. Y. The Williams and Wilkins Company, Baltimore, 1943. IX p., I 1., 708 p. 22 × 28 cm. $13.50.

This is an impressive guide to the internal structure of the human central nervous system as revealed by serial sections. In spite of its being the most complete atlas so far attempted and its large plates (about $7\frac{1}{2} \times 9\frac{1}{2}$ inches), it is not cumbersome. The narrow margins (sometimes only $\frac{1}{4}$ inch) are fully justified from the practical standpoint. Space is also conserved by having the labels placed directly on the structure.

The illustrations are unsurpassed enlarged photographs of Pal-Weigert preparations consisting of 20 levels of the spinal cord in cross-section (5th sacral to 1st cervical, × 14 to 40); 39 cross-section levels of the brain stem, from the lower regions of the pyramidal decussation to the anterior extremity of the caudate nucleus (97 plates, × 3 to 22); 21 levels transverse to the long axis of the cerebral hemispheres and more or less in the frontal plane of the brain stem, from the fourth ventricle and splenium of the corpus callosum posteriorly to the anterior end of the caudate nucleus anteriorly (37 plates, × 3 to 5); 15 horizontal sections of the cerebrum, from the lower level of the midbrain to just above the caudate nucleus (21 plates, × 2 to 5); 18 oblique frontal sections of the brain stem (parallel to the brachium conjunctivum), to well above the corpus striatum and from the optic chiasm to the posterior extremity of the pulvinar (40 plates, × 2 to $5\frac{1}{4}$); and 18 sagittal sections of the entire brain stem and body of the corpus callosum from the mid-sagittal plane to the lateral limits of the thalamus and globus pallidus (39 plates, × 2 to 4).

In many instances the whole section is followed by one or more enlargements of limited regions in order to bring out greater detail and to make possible a more complete labeling without obscuring the anatomy. Thus an exceedingly complete atlas has been achieved, which will fit almost every series, regardless of the plane of section.

The confused methods of labeling sometimes encountered and complained of in the preface, has not been mitigated in all places, because of the style of the symbols (abbreviations) used and insufficient space between adjacent legends. The completeness and conspicuousness of the legends — commendable features as such — are naturally partly responsible for the confusion in places where very limited space is available. A combination of large capital letters with small ones and more compactness within certain designations would have alleviated most of the confusion.

Facing each plate is a key to the symbols used, in Latin terms of the author's selection. Some BNA terms have been used as have also some from the German revision (INA); but new more or less self-explanatory names are also introduced. One may question the advisability of anything short of concerted action of some representative body in the replacing of terms already in use with new ones, for neuroanatomy is already overburdened with synonyms. In fact an International Commission on Anatomical Nomenclature was established in 1936, with various national committees, of which the German, British and American had already done considerable work before being interrupted by the war. Neuroanatomical terminology has long been a sore spot — dating back at least to Stensen (1668), who could see no sense in calling brain structures nates, testes, anus, vulva, penis, etc.

Following the plates is a large section (170 pages) devoted to explanations, giving synonyms, the major subdivision of the nervous system to which each structure belongs, a brief descriptiion, fiber connections and function, as well as the plates in which each structure is identified. Needless to say this glossary is exceedingly valuable. Due to the number of series involved, locating a particular plate would have been expedited by giving the page on which it is found, rather than the number of the section. Incidentally the latter was found not to correspond in two places. Thus on page 543 under Alvx (Alveus extraventricularis) the reference is to Plate T9; 1021, 1071, when there are no such plates. The first plate intended is obviously the one on page 216, which is an enlargement of part of T9-1020 and is so designated. The same discrepancy exists in the reference to T9-1071. A page index to the plates on a detached sheet accompanies the volume; but this intermediate step could have been wholly eliminated by referring directly to the page. Since the general plan of naming structures consists of using a generic term followed by letters representing modifying terms, interpretation of the symbols is facilitated by first learning the list of abbreviations representing this generic classification (pages 530–531).

The explanations are in general comprehensive and up-to-date summaries. The author is a little cautious (page 533) about abandoning the notion that the mesencephalic root of the trigeminal nerve may contribute proprioceptive fibers to the ocular nerves. There is apparently no reference to the presence of mesencephalic root fibers in the so-called motor root of the trigeminal nerve, although that is a common conception. The association of the bulbar portion of the accessory nerve (XI) with the laryngeal musculature is not specified (page 537). The lateral spinothalamic tract is not mentioned (page 610) in connection with the area designated the lateral lemniscus, although this is definitely assured, especially in the region of upper pons and lower midbrain. The thalamus and hypothalamus are conservatively treated, while the rhinencephalon (pages 601, 604, 667 and other places) is given relatively a large amount of space (possibly reflecting the difficulties encountered in teaching this part of the brain to students). Placing the calcar avis (hippocampus minor) in the rhinencephalic segment (page 609) is however hardly justified, nor would it seems advisable to use caput gyri hippocampi as a synonym of cornu Ammonis (hippocampus major) (page 559).

Some difficulty is experienced in identifying certain components of complex fasciculi as listed individually elsewhere, in spite of and/or because of the abundant synonyms given; as, for example, in connection with the medial longitudinal fasciculus (pages 573–574) and tractus vestibulo-mesencephalicus and tractus vestibulo-nuclearis (page 696). This of course is to some extent a reflection of the lack of a standard terminology in the literature and disagreements among authors. Naturally the emphasis would be altered here and there by individual investigators, depending upon their own experience. Thus the reviewer considers that the vestibulo-spinal components of the medial longitudinal fasciculus are somewhat under-rated (page 696), particularly as to the distance they extend caudally, as well as to the contributions received from the triangular (medial) vestibular nucleus (page 620).

The last 6 pages contain nearly 250 selected references, mostly of recent date. The book throughout bears further witness to Doctor Riley's extensive familiarity with brain anatomy, and should meet with a wide reception among investigators of the nervous system. It represents a Herculean task well done. Some structures may appear to have been unnecessarily subdivided; but judging from the past this is the safest course. One need but recall that as late as 1851 W. E. Horner (8th edition of his Special Anatomy and Histology) regarded the attempts to identify and name the cerebral convolutions as being "beyond sound limits of anatomical description". Yet today the 2 to 3 dozen areas then labeled have been doubled and up to 200 are postulated (on the basis of architectonics).

As a complete atlas it lacks parallel series of illustrations from Nissl or other preparations which show positively and more accurately the limits of cell groups; but such a companion volume is already contemplated and will be awaited with equal interest.

It is enlightening to compare this the most recent representation of the inner structure of the hub of the nervous system with one of the earliest attempts at such serial-section portrayal, by Benedict Stilling (1846). Stilling's magnificent lithographs, based on unstained freehand sections of the human brain stem fixed in spirit of wine, are not lacking so much in the number of discernible areas or structures (where there are comparable plates) as in the interpretation of the appearances. Nearly a hundred years of analysis, made possible by the development of the then undreamed of and now almost unbelievabler refinement in technique, may now be read into these intermingling fiber bundles and cell masses.

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STUDIES IN SKULL GROWTH. CORONAL SUTURE FIXATION

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SEVEN FIGURES

Membrane bones of the cranial vault increase in size in two ways: partly by accretion on their external convex surfaces, with resorption on their inner concave surfaces; partly by accretion at their contiguous or sutural borders. An analogous distinction can be made in the growth of the cartilaginous limb bones; but with the important difference that the medullary cavity of the shaft is enclosed within a single bone, and it is quite obvious that enlargement of the marrow cavity is entirely due to resorption, although its increases in diameter and in length are related respectively to the two methods of growth. On the other hand the walls of the cranial cavity are built up of separate units, and enlargement of the cavity is the result of contributions by each unit and by both methods of growth. Both methods are also concerned in the shape and bilateral symmetry of the skull, and in the hereditary growth patterns of different species and breeds.

This arrangement presents a number of problems. What is the relative extent of cranial enlargement by sutural growth and by remodelling? Can either process compensate for deficiency or abnormality of the other? Are both processes affected alike by general hormonal factors, or differently, since enlargement is produced by accretion and by resorption respectively? Do the same local forces of tension act on both? Moreover in sutural growth, there is normally a marked inequality in the rate of growth at different sutures; e. g., direct measurement of the nasal bones of puppies shows that these bones may double in length while their width remains almost unchanged. Also the development of bevelling and of interdigitation suggests that in some cases resorption may be associated with accretion in the mutual interaction at contiguous borders.

Some of the mechanical problems in suture growth can be tested by experiment. The coronal suture presents the most favorable features for experimental interference. A very simple operation has been performed on puppies at the age of about 3 weeks. It consisted in cutting a circular disc of bone, of which part of the coronal suture

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formed a diameter or major chord. A cork borer (1.5 cm. diameter) was used instead of a trephine, in order to minimize the amount of bone lost, and to obtain a disc with slightly bevelled edges which would fit tightly in its hole. The periosteum was as little disturbed as possible. The disc was then rotated in situ, without completely detaching it from its dural attachment and blood supply along the suture (although the vessels might be obstructed by the twist), until the disc portion of the suture lay at an angle of about 90° to the rest of the suture. The disc in this new position was then pressed firmly and evenly into its hole. Since the disc is in two halves, with a sutural hinge, it is readily adapted to the curvature of the skull.

Two results were anticipated from this operation: (a) that bony union would occur at the cut edges round the whole circumference of the disc, firmly binding the frontal and parietal bones, and thus preventing growth and movement at the coronal suture on the operated side, with consequent asymmetric deformity of the skull, and probably synostosis of the whole immobilized suture; (b) that growth would continue at least for a time in the rotated part of the suture, with resultant buckling and elevation of the two halves of the disc, since they could not spread laterally in their own plane.

Actually neither of these things happened in any of the five puppies on which this first operation was performed. In three of the same litter, operated upon when 3 weeks old and killed 5 weeks later, about one-half of the circumference of the disc had become knit to the edges of the hole by bony union; the other half remained fibrous and unossified, constituting a new artificial suture, and serving to re-establish the continuity of the coronal suture. Consequently the frontal and parietal bones were not rigidly united, and there was no impediment to normal expansion of the skull. In one of these puppies (no. 18) the new suture line followed the anterior border of the disc; in another (no. 20) the posterior border; in the third (no. 19, fig. 1) it followed a devious zigzag course, first along the upper posterior quadrant, then along the rotated portion of the original suture, then by the lower anterior quadrant to reach the lateral part of the coronal suture. In all three animals, the rotated portion of the suture remained open, i. e., fibrous, and some growth might have continued in it. Each disc does show a slight convexity, but this appears rather to be associated with the fact that the disc is surrounded by a smooth circular depression, formed where the periosteum was cut and had retracted. An area of skull denuded of its periosteum undergoes some surface resorption, and

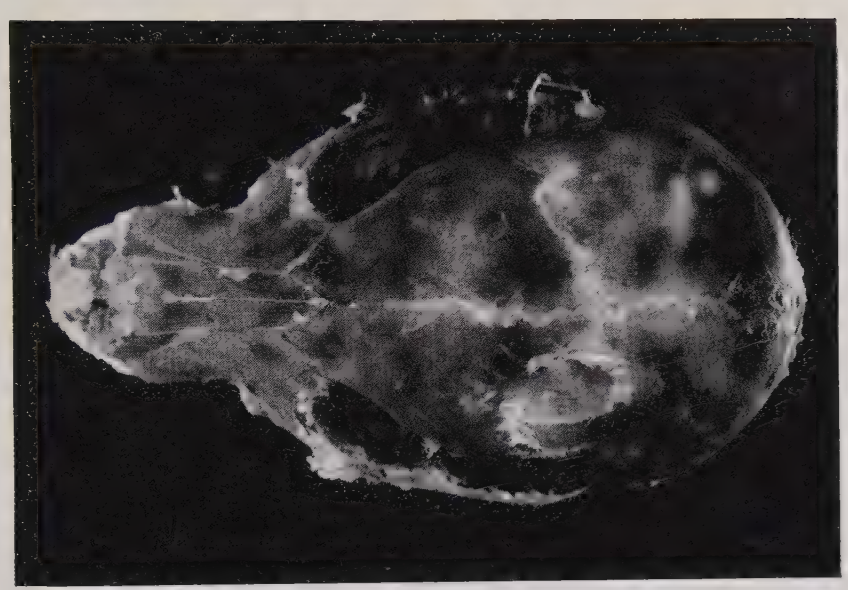

Figure 1

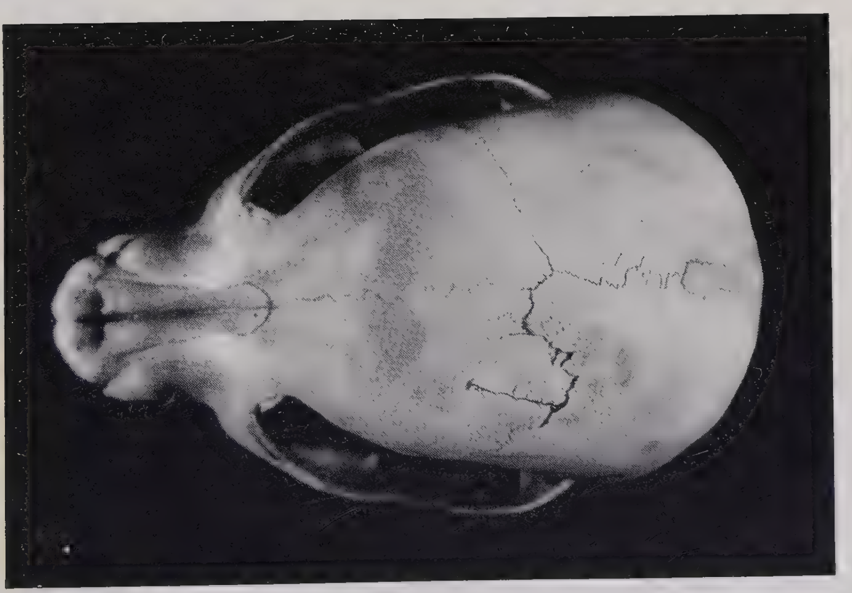

Figure 2

hollows in the skulls of growing puppies produced in this way often persist for months.

Two puppies of another litter were operated upon when 25 days old. In one (no. 48, fig. 2), killed 6 weeks later, a new suture line has been established round the posterior border of the disc; the anterior border is smoothly knit to the frontal bone; the rotated part of the suture remains open on the outer surface, but is fused on the cerebral surface. Growth of the skull has been normal. The other puppy (no. 49, fig. 3) was killed after 16 weeks. The parietal segment of the disc, which had been rotated to the medial position, is smoothly united to the frontal bone, and the frontal segment, now lying postero-lateral, to the parietal bone. Both coronal sutures are fused on the cerebral surface, but on the outer surface are partly open, and it can be seen that continuity of the left (operated) coronal suture had been temporarily established along a zigzag course as in puppy no. 19. The suture edges are bevelled, the posterior overriding the anterior, and this has occurred also along the rotated part of the suture, the posterolateral (originally frontal) segment overlapping the medial. The line of the sagittal suture and the interparietal bone form an angle of 170° with the line of the frontal and internasal sutures, and also the skull is twisted, as described below; but measurements of the right and left sides of the skull are practically identical.

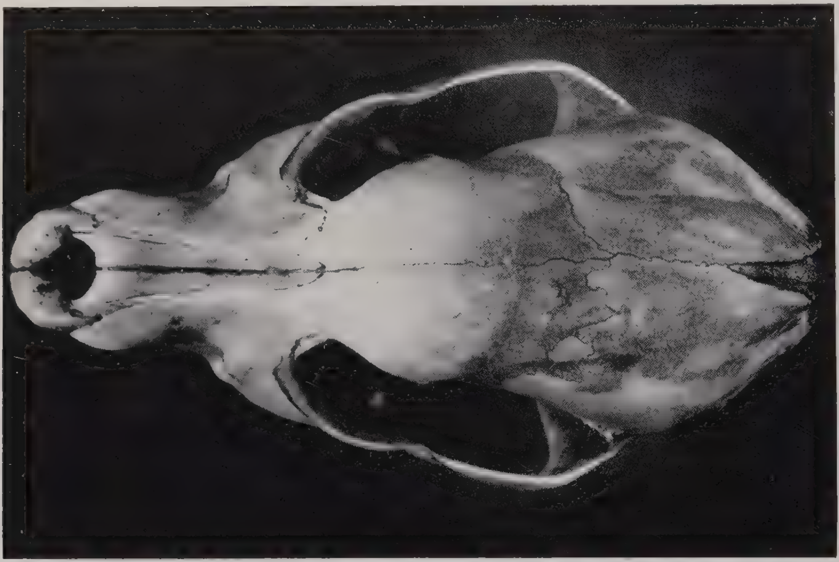

Figure 3

The conclusion which may be drawn from these experiments with the rotation in situ of a bone disc cut across the coronal suture, in contrast with the results described below with insertion of solid discs, is that the presence of a sutural division in the disc contributes to the maintenance of normal mobility and growth in the affected suture.

A modified form of this operation was performed on three puppies when 20 to 25 days old, and caused a greater disturbance of skull growth. Two discs were cut out, one from the left coronal suture, the other from the right parietal bone. The two discs were detached and interchanged, the solid parietal disc being inserted into the sutural hole, and the hinged coronal disc into the parietal hole, the transplanted portion of the left coronal suture being placed approximately parallel with the right coronal suture. One pup (no. 50, fig. 4) was killed after 12 weeks. Both discs have united smoothly and completely around their circumference. In the coronal disc inserted into the parietal bone, the suture remains open on the outer surface only; the smoothly convex disc forms a slight prominence above the contour of the parietal. Continuity of the left coronal suture has been interrupted, the solid disc forming a bridge of bone joining frontal and parietal bones. The suture both medial and lateral to the disc is almost completely fused, whereas the right coronal is still fully open. This fixation of the left coronal has resulted in a lateral curvature of the skull,

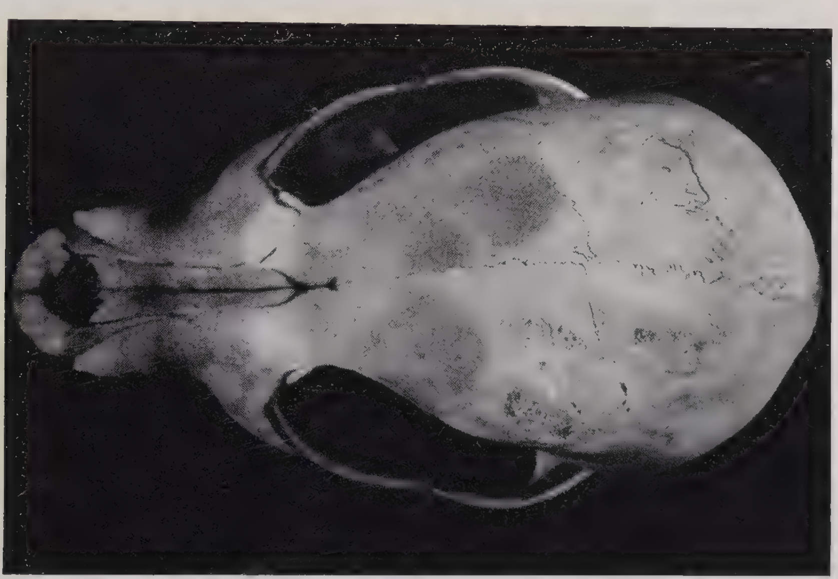

Figure 4

not however of sufficient extent to be noticed while the dog was alive. The sagittal and frontal sutures form an angle of 166° at the bregma. The squamous suture and the zygomatic arch on the left side are 2 mm. shorter than those on the right. The lateral circumference from nasion to inion is 5 mm. shorter on the left side.

In another puppy of a different litter (no. 58, fig. 5), which was killed after 11 weeks, the results and comparative measurements very closely resemble those of no. 50. Sagittal and frontal sutures form an angle of 170°. The left squamous suture and the zygomatic arch are 2.5 mm. shorter, the lateral circumference 4 mm. shorter than those on the right.

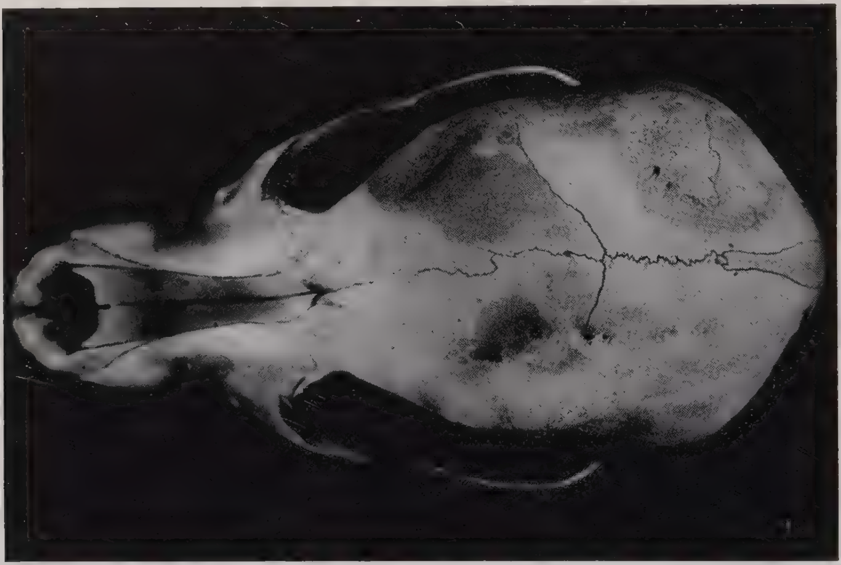

Figure 5

The third puppy (no. 56, fig. 6) was killed 14 weeks after operation. Both discs are fused smoothly in the skull. The left coronal suture is almost completely fused, the right is still open on the outer surface only. Sagittal and frontal sutures form an angle of 170°. The squamous sutures are irregular. The left lateral circumference is 6 mm. shorter than the right, the left zygomatic arch 2.5 mm. shorter. As in the other two skulls, the shortness of the left zygomatic arch is due almost entirely to diminished growth of the malar bone.

As can be seen in the photographs, the part of the left coronal medial to the transposed disc has retained its early direction almost

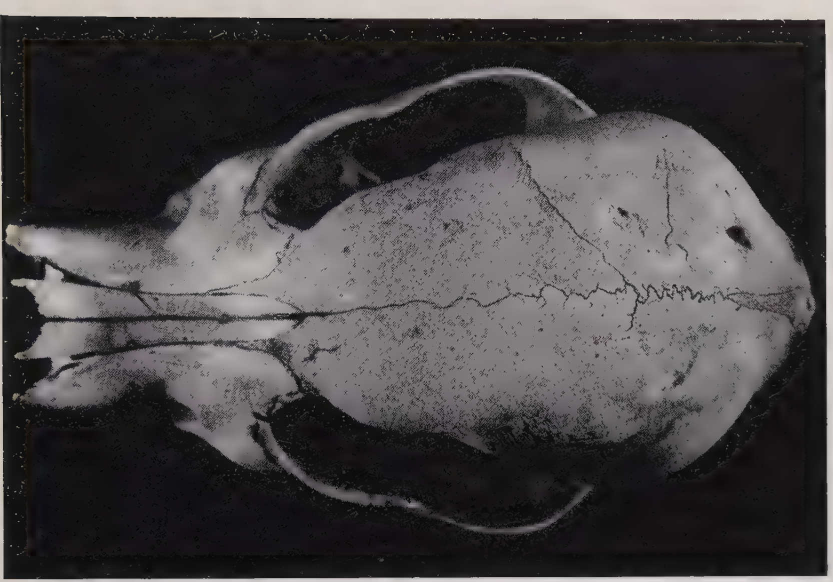

Figure 6

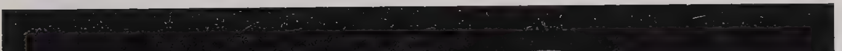

Figure 7

at right angles to the midline, whereas the right coronal has acquired the usual acute forward slant.

An additional feature which is present in these three skulls with fused left coronals, and also in no. 49, is that the anterior half of the skull is twisted downwards on the left side in relation to the posterior half, so that (the mandible having been removed) they do not stand evenly on the table; when the two mastoid bullae and auditory meatus are horizontal, the teeth and alveolar margins and the lower margin of the orbit on the left side are 1 to 2 mm. lower than on the right. It was not noted whether malocclusion of the teeth was present.

In one puppy (no. 55, fig. 7) a single disc was cut involving the anterior part of the frontal suture; the disc was rotated without being detached. The operation caused considerable hemorrhage. This puppy was killed 14 weeks later. The disc has become smoothly fused around its edges, but its center has become thinned and partly perforated by resorption owing to periosteal damage. The position of the disc is now relatively more posterior, due to the normal active growth at the anterior border of the frontal bones. The only effect of the operation has been to cause a localized premature fusion of the frontal suture. To determine whether this would induce any change of shape in the dog skull would require a number of controls in litters of stable breed, which have not been available to us.

DISCUSSION

Growth and moulding of the skull, considered as a mechanical process, involves progressive changes in the actual and proportional size, the shape, and the relative position of its constituent bones. In the expansion of the cranial cavity, in addition to the action of internal tension, there is therefore a mutual interplay of stresses between its different parts. Growth at any single point would be impossible if the rest of the skull were rigid. It would appear to be a function of the sutures between bones, in remaining ununited throughout growth of the skull, not only to contribute in various degrees to increase in size and change of shape of these bones, but especially to permit of the relative displacements which necessarily occur between the unequally growing parts, and at the same time to prevent dislocation or separation by external forces, including the pull of attached muscles; i. e., to act as membranous joints, capable of the slow gradual angular movements imposed by growth at other points. (Mijsberg ('32) indeed claims that provision for such flexions is the main or only function of sutures.) For example, the early fusion of the human metopic suture implies not only arrest of growth at the medial borders of the two frontal bones, but also absence of flexion between their planes (apart from changes of contour by remodelling). In the dog skull, growth is minimal in the midline (Giblin and Alley, '42), yet the frontal and sagittal sutures remain open, and evidently function as a hinge to allow broadening of the skull by growth at its base; while the dovetailed interdigitations in the sagittal and parieto-interparietal sutures resist the separative pull of the temporal muscles. Bevelling of the coronal, which may be reversed in its different parts, maintains firm coaptation during continued growth, as also does the tenon and mortise junction of the malar and maxilla. The oblique scarfing of the temporo-malar joint gives many times the strength of a square abutment, and its curved and partly stepped interface prevents sideslip or override; in the skulls which have the left side shorter, there has been diminished growth at this point, not increased overlap. Actual growth and resorption at a suture by varied extent in different parts of it may also provide for and accompany relative displacements, e. g., the changes and variations of direction and form in the coronal, and of the amount of overlap and the inclination of the interface in the squamosal. Brash ('34) after feeding with madder was unable to detect any new bone deposition on the sutural faces. He concluded that remodelling on the free surfaces is the only process occurring in the membranous bones of the growing skull, and alone must account for all changes of size and shape and relation, including the shifting of suture lines at bevelled sutures. But the madder method has limitations, and certainly growth at sutures occurs and can be demonstrated by other means (Giblin and Alley, '42).

Suture growth is by extension of the ossified border of one or both of the contiguous bones; for this bone growth to continue, there must also be pari passu continued formation of more membranous tissue; or perhaps in some cases there is extension of one bone margin only and an equal resorption of the other, producing either interdigitations or a creeping migration of the whole suture, with shifting of the axis of flexion. The nature and extent of growth are determined by the mutual forces arising from simultaneous growth in other parts. In the distorted skulls described in this paper, growth in all parts shows a smooth and harmonious adaptation to the retrictions imposed by a localized fixation. Virchow (1856) observed that a premature synostosis induced a series of secondary and compensatory changes, including local overgrowths and shortenings, and fusion of other sutures. Brash('29, p. 239) states that "asymmetry, even if related to some local circumstance, can nearly always be shown to extend throughout many details of the structure of the skull,'' and quotes Darwin's principle of ''correlation of growth,'' but is dubious as to the mechanism.

Sutures fuse by complete ossification of the membranous tissue. A primary condition for this to occur (given normal hormonal and nutritional factors) appears to be absence of movement; compare the union of fractures, and the synostosis of immobolized joints. In the case of most of the cranial sutures, there must be cessation of growth in all parts of the skull before they can fuse. Conversely, if premature fusion occurs at any point, it will tend to spread progressively due to the rigidity produced. Bolk ('15) in a series of young human skulls found that premature closure of sutures was frequently multiple, but ascribed this to an assumed general tendency. In our skulls, fixation at one point of one coronal has resulted in the rapid fusion of other parts of that suture; the fact that skull growth continued, though in modified form, can be ascribed to the high growth activity and plasticity of young skulls.

CONCLUSIONS

1. Premature fusion of a suture can be induced by insertion of a solid bone bridge.
2. Growth of the whole skull is plastic, i. e., growth at any point is adapted to growth at other points by interplay of stresses; disturbance of growth at one point affects the rate and direction of growth of other parts.
3. This plasticity does not prevent asymmetry; adaptations to a localized inequality of growth may produce abnormal curvatures and torsions.

The expenses of this research were met by a grant from the Blanche E. Hutchinson Research Fund of McGill University. We have been much indebted to Prof. B. P. Babkin for his continued active interest and helpful suggestions.

SUMMARY

An operation to interfere with normal growth at one coronal suture was performed on young puppies. A disc including a portion of the suture was trephined, and either rotated in situ or replaced by a disc of solid bone. In the latter case there resulted premature synosotsis of the suture and asymmetric deformation of the skull. The rôle of sutures in skull growth is discussed.

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A CASE OF PSEUDO-MONOVENTRICULAR HEART TERMINATING IN BRAIN ABSCESSES

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TWO FIGURES

INTRODUCTION

Congenital defects of the interventricular septum have been of interest to the physician not only because of the frequency of their occurrence among cardiac anomalies, but also because of the occasional viability of patients possessing them. White and Sprague ('29) have reported one case of Fallot's Tetralogy in which the patient attained the age of 59 years, while others have been known to survive until their early and middle thirties (Baumgartner and Abbott, '29).

A further importance has been attached to such congenital anomalies in that brain abscesses frequently occur as a terminal illness in such cases.

The following case has been of especial interest to us in that it presents not only an extensive interventricular septal defect, but is one in which the terminal illness resulted from bilateral cerebral abscesses. The heart described in this paper was obtained at post mortum and presented to the Anatomy Department as an embryological specimen.

CLINICAL NOTES

Incidental clinical notes indicate that the heart was that of an 8-year-old male. Physical examination at the time of admission to a hospital (Rex Hospital, Raleigh, N. C.) revealed that the patient was suffering from pharyngeal abscesses. On examination of the chest the cardiac rhythm was found to be regular, a systolic thrill was palpable over the precordium, and a loud, blowing systolic murmur could be heard over the entire heart.

The fingers were clubbed and there was a history of blueing about the ears and nails on exertion. However, the patient as a baby had not been blue.

The autopsy findings and anatomical diagnosis were: (1) abscess of each frontal lobe of the brain and (2) congenital absence of the interventricular septum of the heart.

ANATOMICAL DESCRIPTION OF THE HEART

In external appearances, the heart differs but slightly from that of a normal child of 8 years. A marked disproportion exists in the size of the right and left auricular appendages, the left being considerably smaller than normal. The ventricular surfaces below and to the left of the right atrium in the region usually occupied by the conus arteriosus is somewhat flattened. There is a transposition of the great vessels leaving the heart, as the aorta arises in front of the pulmonary artery.

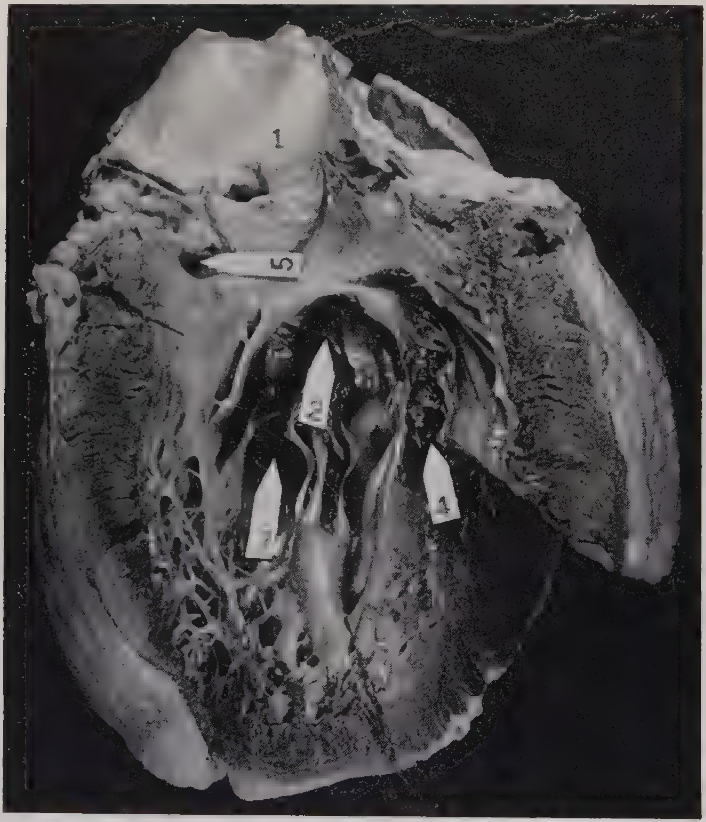

Fig. 1 The heart with the ventricle and aorta incised to show the position of the five openings into the common ventricle: (1) Aorta, (2) Pulmonary, (3) Tricuspid, (4) Bicuspid, (5) To vestigial right ventricle.

Inspection of the inner surfaces of the auricles reveals a normal septum without defect. The coronary sinus and other afferent vessels were found to enter in the usual locations.

Incision of the ventricular wall discloses one large ventricular cavity with five foramina. These foramina are, the right and left atrioventricular orifices, the pulmonary and aortic orifices, and an opening which on further dissection appears to communicate with a vestigial right ventricle (fig. 1).

The atrioventricular orifices are guarded by the bicuspid and tricuspid leaflets and appear to be normal in all respects. The aortic and pulmonary orifices and valves are, with the exception of an apparent pulmonary stenosis, normal in appearance. The right and left coronary arteries arise behind the two posterior aortic cusps and may be traced to a normal distribution.

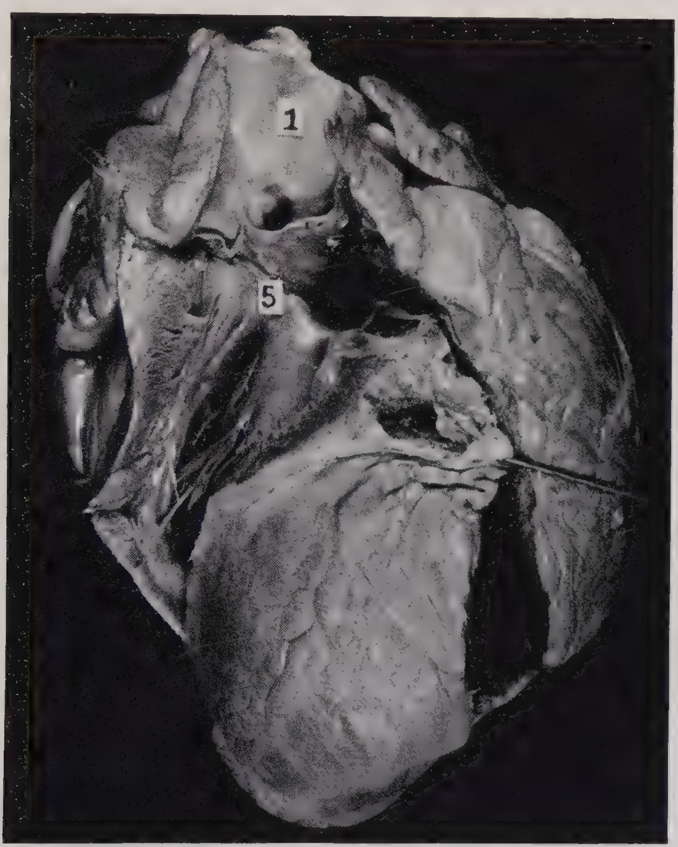

Fig. 2 Heart with the myocardium reflected to show the position of the vestigial right ventricle: (1) Aorta, (5) Opening to vestigial right ventricle.

Just below the anterior aortic cusp and above the annulus fibrosus is seen the fifth opening which is approximately 7 mm. in diameter and leads to a slit-like vestige of the right ventricle (fig. 2). This ventricle is situated in the position normally occupied by the conus arteriosus.

The cavity of the ventricle is approximately 6 cm. long and is lined with endocardium. The cavity is compressed between the adjacent layers of myocardium, the superficial layer of which is about 4 mm. and the deep 1 cm. in thickness. This vestigial space is found to have no other communication with the other chambers of the heart.

DISCUSSION

Defects involving the interventricular septum of the heart are not infrequent. According to Carpenter ('09), the incomplete interventricular septum is one of the most commonly occurring anomalies of the heart and is frequently associated with stenosis and atresia of the pulmonary artery, transposition of the great vessels, and occlusion of the tricuspid orifice. Certain aspects of the heart described in this paper resemble those general conditions as stated by Carpenter in as much as there was a definite transposition of the great vessels and stenosis of the pulmonary orifice.

Physiologically this heart may be classified as a monoventricular heart. From the morphological standpoint, most of the criteria necessary for the fulfillment of the "tetralogy of Fallot" are present; however, certain differences are apparent. If one compares the description of this heart with the chief characteristics as set forth by Abbott ('36) for this type of anomaly the following variations are noted: Generally the right ventricle is hypertrophied while in this case the right ventricle, in reality only a blind pouch, was functionally non-existent. Usually the entire pulmonary tract is hypoplastic and the pulmonary valve is bicuspid. A careful examination of our specimen reveals that the pulmonary valve is normal and that only the pulmonary orifice shows stenosis.

Several theories, based upon embryological and physiological data, have been proposed to explain some of the various conditions observed to occur in the morphology of the heart. Ngai ('35) in describing a heart with a defective ventricular septum in which both pulmonary and aorta arose from a small right ventricle suggests that this ventricle resembled the embryonic bulbus cordis. In support of this view, Ngai suggests that the bulbo-ventricular septum shifted ventrally and became the interventricular septum. If such were the case the small size of the right ventricle would be related to the failure of the septum to shift far enough thus producing a ventricle composed entirely of the bulbus cordis. Such an explanation does not readily lend itself to the heart described in this paper for the aorta and pulmonary arteries arise from the common left ventricle rather than the vestigal right.

Further objection to this theory is to be found in the work of Frazer ('17) who states that the disappearance of the bulbo-ventricular ridge is necessary for the formation of the aortic vestibule.

Spitzer ('23) expresses the opinion that many heart defects may be traced to a failure of the heart to rotate in a clockwise fashion during the course of its development. Since this rotation may condition the association of the pulmonary and aortic trunks with the right and left ventricles and the alignment of the various components of the interventricular septum its absence might be responsible for the condition found in this heart.

In the case of the heart described in this paper, all components of the interventricular septum are present but the septum is incomplete and perforated in the region normally occupied by the pars membranacea. According to Frazer the pars membranacea is formed by the fused auriculo-ventricular cushions, the auriculo-vestibular portion of the right and of the upper cushion and the interventricular part of the lower which becomes associated with the interventricular septum proper. If these components are not brought into reasonable proximity by rotation closure is not complete. Rosedale ('35) also suggests this theory as an explanation of dextroposition of the aorta and septal defects by stating that the shift in aorta-pulmonary relations so changed the arrangement of the septal constituents that they failed to fuse.

The extreme asymmetry of the two ventricles is probably conditioned by physiological factors. Since the lack of rotation allowed both the right and left auricles to communicate with the common left ventricle, and both arterial trunks exit from the same chamber, the difference in functional activity could be responsible for the variation in volume. The activity of the right ventricle was extremely limited as the only means by which blood might enter and leave its chamber was by way of the small foramen at the base of the aortic vestibule.

This case is of further interest in as much as it presents a patient with a cardiac anomaly whose terminal illness resulted from brain abscesses. A careful examination of the heart reveals no indication of endocarditis, and at autopsy no lung abscesses were found. It may be assumed, therefore, that the path of infection might have led from the primary locus of infection directly through the heart to the cerebral lobes. Hanna ('41) summarized the literature up to 1941 and found that twenty-three known cases of congenital cardiac anomaly had been complicated by brain abscesses. Of these, sixteen were associated with interventricular defects, one with both interventricular and interauricular septal defect, and the remaining six foramen ovale. The case reported in this paper brings the known total to twenty-four.

SUMMARY

A pseudo-monoventricular heart has been described and the differences between this anomaly and the "tetralogy of Fallot" have been cited and discussed.

The terminal illness, which not infrequently accompanies this group of cardiac anomalies, was cerebral abscess.

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A CYTOLOGICAL STUDY OF STORAGE AND SECRETION IN THE DEVELOPING LIVER OF THE MOUSE[1]

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TWO PLATES (TWELVE FIGURES)

The liver of the fetal mammal performs many functions, including the storage of glycogen and fat and the secretion of bile pigments (Feldman, '20; Stieve and Kaps, '37). After birth the liver undergoes profound changes in environment: the blood, formerly supplied by the umbilical veins and richly oxygenated, is now derived primarily from the portal vein, with but a small amount from the hepatic artery; the oxygen tension of the blood bathing the liver cells is now quite low, and the blood flow is sluggish. Moreover a diurnal rhythm of eating is gradually adopted, so that the supply of food materials to the liver is no longer continuous.

The fact that the liver cells are thus faced with a new environment after parturition suggested that the reorganization of the functioning of the cells, the possible adoption of new activities, and the development of cyclic processes in the liver might elucidate the relation of the cell organelles to the processes of storage and secretion. A series of young mice was therefore studied.

I wish to thank Prof. J. Walter Wilson for his helpful criticism during the course of the investigation.

MATERIAL AND METHODS

White mice of the Swiss strain served as the experimental animals. The number of animals in each litter was reduced to six at the time of birth so that the remaining young were well fed. The animals were weaned at 4 weeks of age and fed on Rockland Mouse Pellets thereafter. For this developmental series only large, healthy-looking individuals were selected.

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Brown University, September, 1943.

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The mice used were 1, 3, 6, 9, 13, 18, 24, 33, and 45 days of age; a male and a female of each age were sacrificed. All of the animals were killed at approximately 5 P.M.

The same histological methods were adopted as in the previous study (Deane, '44): (1) The Bouin-Delafield method for a study of the general appearance of the tissue; (2) a modified Regaud-Heidenhain method for demonstrating mitochondria; (3) the Champy-Kolatchev method for impregnating the Golgi substance; (4) the Forsgren method for bile acids; (5) the Bauer-Feulgen method for demonstrating glycogen; and (6) a formol-sudan IV method for staining neutral fats.

Judgments of the quantity of bile acids, of glycogen, and of fat have been made for each animal studied. The symbol (+) indicates that only scattered cells in the lobule contain the substance in question; one or more +'s indicate that an appreciable quantity is present. An attempt has been made to judge the amount of the material in the lobule as a whole, irrespective of whether it is localized in a zone or is distributed evenly in the lobule. It is presumed that these judgments may serve as an approximate measure of the amount of the substance that could be ascertained by biochemical analysis (Deane, '44).

OBSERVATIONS[4]

The mouse liver, at the time of birth, is composed about equally of liver parenchyma and hemopoietic tissue. The blood-forming tissue quickly decreases in amount and disappears between the ninth and thirteenth days. For the first week the liver lobule cannot be discerned since the tissue is so unorganized, but by the ninth day the relationships of the veins and the trabeculae are apparent.

In this series of mice, mitosis of the hepatic cells persists through the twenty-fourth day. Although the liver cells are small and uniform in size at birth, they are much larger and more variable in size in the older animals (compare figs. 5 and 7).

The mitochondria in the 1- and 3-day animals have the same form in all of the cells; no matter what the relationship of the cell to the veins they are rather heavy rods and not very crowded in the cytoplasm (fig. 1). From the sixth day on, however, a variation of the mitochondria in the lobule develops. They now have the form of spheres and short rods in the peripheral zone (figs. 2 and 4), elongated rods in the middle zone, and long rods of filaments in the central zone (fig. 3). By the ninth day the mitochondria of the inner zones are also finer than in the peripheral, and at 18 days those of the most central zone become quite tenuous. From the ninth to the thirty-third day those of the peripheral zone appear not only larger than those of the more central regions, but swollen (fig. 2). After 24 days, the mitochondria of the central zone are light in their staining reaction (less siderophilic) and are sometimes fragmented in the cells adjacent to the central vein.

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[4] Detailed observations are included in the thesis which is available at the John Hay Library, Brown University.

During the first week, isolated cells are undergoing chondriolysis (fig. 1) and others are completely blackened with the hematoxylin. The cytoplasm of all of the cells retains a good deal more of the stain than is the case in older animals. The abnormal cell types disappear after the eighteenth day and the cells become uniform in their staining reaction.

The Golgi substance is neither large nor easily impregnated in the younger animals. During the first 3 weeks, although the cytoplasm of the hepatic cells is exceedingly osmiophilic, the Golgi material is not impregnated by less than 3 weeks' treatment with osmic acid. From the first to the thirteenth day it is composed of vesicles and clusters of small granules lying near the nucleus and along the bile capillary (fig. 5); on the eighteenth day the granules have coalesced into strands and networks (fig. 6); and from the twenty-fourth day on the Golgi substance is composed of broken networks which lie across the cell from the nucleus to the bile capillary (fig. 7). The strands of the networks are both finer and more lightly impregnated at the center of the lobule than at the periphery.

The amounts of the products of cell activity — bile acids, glycogen, and fat — have been judged and they are recorded in the following table.

Bile acids are not demonstrable in any quantity until the sixth day, when a few granules appear in the peripheral cells of the lobule. They gradually become more abundant and reach the more central part of the lobule (fig. 8).

Some glycogen is present in the liver cells on the first day and it increases quite steadily in amount until the eighteenth. At first it is most concentrated in the center of the lobule (fig. 9), then it becomes evenly distributed (fig. 10). In the weaned animals very little is being stored by the liver at 5 P.M. and when present, it is distributed evenly or centrally in the lobule.

Fat fills the liver cells at the time of birth (fig. 11). The amount decreases regularly (fig. 12) and has virtually disappeared by the fourth week. Immediately after birth the fat is evenly distributed among the cells of the lobule. It disappears first from the central region, from 18 days on, there is a small amount of fat present which may be centrally or irregularly distributed.

TABLE 1

Bile acid, glycogen and fat content of liver in histological preparations.

AGE, IN DAYS	SEX	BILE ACIDS	GLYCOGEN	FAT
1	♂	0	+	++++++
	♀	0	+	++++++++
3	♂	0	+	+++
	♀	0	++	+++++
6	♂	+	++++	++
	♀	++	+++	++
9	♂	+++	+++	++(+)
	♀	+++	+++	++
13	♂	+++	++	+++++
	♀	+++	+++	+++
18	♂	++++	....	++
19	♀	+++	+++++	+
24	♂	+++++	++	0
	♀	+++++	++	0
33	♂	++++++	+	0
	♀	++++++	0	0
45	♂	++++++	+	0
	♀	+++++++	0	+

DISCUSSION

The liver of the mouse at birth still contains a considerable amount of hemopoietic tissue. Although this observation has been made previously in the case of the mouse (Jacobj, '25), it is generally stated that hemopoiesis is discontinued in the mammalian liver before parturition. The nests of blood-forming tissue have virtually disappeared by the end of the second week, and after this time the liver lobule, as it exists in the adult, is readily discernible.

Growth of the liver parenchyma appears to take place in two ways: first, by mitosis, which continues through the twenty-fourth day in this series; second, by a growth of the cells. At birth, all of the cells are small and quite uniform in size. They gradually increase in size and some become very large. This change in cell size is accompanied by an enlargement of the nuclei, which may indicate a multiplication in the number of chromosome complements in the cells (see Jacobj, '25; Beams and King, '42; Wilson, '42).

The ability of the liver cells to store glycogen originates early in the fetal liver and in the rabbit is already maximal at the time of birth, according to Stieve and Kaps ('37); the actual amount of glycogen present in the liver cells falls off during parturition but is soon restored (Mitani, '35). In the present series, the amount of glycogen is fairly small on the first day but rises quickly and steadily until the eighteenth day. Such a rise has been reported also by Seckel and Kato ('38) and by Heymann and Modic ('39), although the former authors found next to none present before the third week of post-natal life. After the eighteenth day, very little glycogen is to be found in the livers: by this time the animals have adopted a diurnal periodicity in eating, and at 5 P.M. the amount of glycogen still present is very low as shown in an uncontrolled diurnal-cycle series of adult mice (Deane, '44). There is a certain amount of variation between the animals in my series, but this is to be expected since no attempt was made to kill them at any constant time after feeding.

The fetal liver likewise stores fat; the amount of fat reaches a maximum at the time of parturition (Stieve and Kaps, '37). In the liver of the day-old mouse, it is heavily concentrated (fig. 11); it decreases gradually and fairly steadily (fig. 12), disappearing most rapidly from the central region of the lobule. Although no explanation for this change in distribution has been found, the description is in accord with that of Rice and Jackson ('34) and that of Stieve and Kaps ('37). After the animals have begun to eat prepared food, however, any fat present in the liver cells is located in the central region as is characteristic for the adults on this diet. It is believed that the location of the fat is dependent upon the low oxygen tension of the central part of the lobule, which prevents the rapid oxidation or phosphorylation of the fats supplied to the cells (Deane, '44).

Bile acids do not appear to be secreted in any significant quantity before the sixth day of post-natal life. This finding seems to be in agreement with biochemical analyses, which show that the bile of infants contains very little bile acid material (Feldman, '20). The quantity secreted by the cells then rises (fig. 8). The increase with age may be explained by the fact that a portion of the bile acids is resorbed and resecreted so that the amount present in the cells is somewhat cumulative (Josephson, '41).

Seckel and Kato ('38), in a study of the development of liver function in the rat, however, stated that in newborn animals there is a large secretion of bile acids and no storage of glycogen. They then described a general fall in the amount of bile acid material in the liver cells with age and a simultaneous appearance of glycogen. Reciprocal diurnal cycles in secretion and storage were observed to develop during the third week. The authors interpreted these facts as being due to an antagonism between the process of secretion of bile acids and that of the storage of glycogen, such as had been suggested by Forsgren ('28). It is difficult to explain the discrepancy in our observations. Seckel and Kato had used both a histological and a biochemical method for determining the amount of glycogen and the Forsgren technique for demonstrating bile acids. In bile acid preparations it is often difficult to differentiate the granules from the cytoplasm, and a study of their illustrations suggests the possibility that they may not have been observing true bile acid granules. Why their glycogen determinations should have been so low, however, is not clear. In biochemical analyses, one contributing factor to the small amount of glycogen in the livers of the youngest animals may have been the large amount of hemopoietic tissue present. Even though the parenchymal cells did contain some glycogen, the percentage of glycogen for the tissue weight would be very low when compared to the results for older livers in which no blood-forming tissue was present.

The mitochondrial preparations show that, at birth, there is no functional lobulation in the liver. In all the cells, no matter what their position in relation to the veins, the chondriosomes are alike in form and size, appearing as heavy, smooth rods (figs. 1 and 2). This observation agrees with that of Chaves ('20) for the newborn hedgehog, that of Smith ('31) for the newborn rat, and that of Dalton ('34) for the newly hatched chick. By the sixth day, the characteristic intralobular variation of the mitochondria is beginning to appear. At first there is merely a change in form: the mitochondria at the periphery of the lobule are shortened in comparison to those at the center. (Smith ('31) also reported enspherulation of the mitochondria with age, although she observed no zonation in the rat liver.) Later there is also a variation in the diameter of the mitochondria: those at the periphery remain broad and heavy (fig. 4), while those in the more central regions become progressively finer. After the time of weaning, the chondriosomes in the center of the lobule become light-staining and often fragmented (fig. 3). This zonation in mitochondrial form and staining reactions corresponds to that found in the adult mouse (Deane, '44).

As in the diurnal-cycle experiment, the variations in mitochondrial form bear no apparent relation to the changes going on in glycogen and fat storage. They seem to reflect chiefly the effect of a changed blood supply on the liver cells: the blood of the fetal liver is highly oxygenated and flows through the organ quickly; after birth, the greatest amount of blood is supplied by the portal vein and is only poorly oxygenated. Since the blood-flow is sluggish as well, the cells at the periphery of the lobule receive a much greater supply of oxygen than do those at the center (McMichael, '37). There is likewise a greater supply of food-stuffs at the periphery. These differentials in oxygen tension and in food supply within the lobule may be the most important factors in the zonation of the mitochondria.

It is, however, possible that there may be some direct relationship between the changes in the mitochondria and the secretion of bile acids. This is suggested since the ensperulation of the mitochondria occurs at the same time that the secretion of bile begins — on the sixth day of post-natal life. It may well be that the short and relatively heavy mitochondria, characteristic of the peripheral zone of the lobule, are so shaped because of an association with the secretion of bile acids. Both Berg ('20) and Noël ('23) have previously suggested that the mitochondria may be active in such secretion. The kind of evidence produced by my experiment, however, can hardly be considered conclusive.

There are several kinds of unusual cells to be found in the mitochondrial preparations of the youngest livers. In some there are indications of chondriolysis (fig. 1). Others are blackened so that the mitochondria, if present, are not distinguishable. It has been proposed earlier (Deane, '42) that both of these appearances may indicate cell injury. The handling of the young livers may of course have caused the changes, but it seems more probable, because of the regularity of the occurrence in the different specimens, that some abnormal condition exists in the liver cells before the sacrifice of the animal. It might be that the change in the environment of the liver cells at birth provides a stress — in this case, the weaker cells would become necrobiotic and some might even die. (Dalton ('34) described dying dark cells in the livers of newly hatched chicks.) The unusual types of cells disappear after a few days, which suggests that the abnormal cells have died or become accommodated to the new milieu.

The Golgi substance, in this series of young mice, is first demonstrable as small, peripherally located granules, some of which are vesiculated (fig. 5). Since the Golgi substance of the young liver cell was difficult to impregnate, it seems possible that even after 3 weeks' treatment with osmic acid the whole of the Golgi material was not demonstrated. This suggestion is made because Kolmer ('16), Pascual ('16), and Bowen ('26) have all stated that the Golgi apparatus of the liver cell of the young mammal is in the form of a juxtanuclear net. All of these investigators admitted, however, that they had difficulty in demonstrating the Golgi substance of the liver cell, and there remains the possibility that they may have been observing unusual cells.

The granules of Golgi material become fused into tight networks by the eighteenth day (fig. 6), and these in turn ramify and fragment in the older animals (fig. 7). The nets lie both juxtanuclearly and along the bile capillary as is characteristic of the adult mouse (Deane, '44). The Golgi material increases with age not only in volume but also in osmiophily.

If, as described by Pascual ('24), the Golgi material in the fetal liver cell is indeed in the form of a continuous network, perhaps its fragmented condition immediately after birth indicates great secretory activity of the cells. It is generally assumed that the Golgi apparatus in the liver cell is concerned with the segregation and excretion of bile pigments (Cramer and Ludford, '26); and according to Needham ('31, p. 1373) there is a large excretion of bile pigments immediately following parturition. Since the Golgi substance often exists in a fragmented condition at the peak of secretion (Hirsch, '39), the heightened production of bile pigments may serve to explain the form.

After the time of weaning, the Golgi material is more osmiophilic in the peripheral part of the lobule than at the center. It was suggested previously (Deane, '44) that this might result from the decreasing oxygen tension in the lobule from the periphery toward the center.

As in the investigation of the liver during the diurnal cycle (Deane, '44, no constant or absolute relationship between changes in the mitochondria or the Golgi apparatus and the processes of storage of glycogen and fat and of secretion of bile acids is apparent. The development with age of zonation in the form and appearance of the mitochondria and the Golgi net and also in the amount of storage and secretion reemphasizes the earlier conclusion that all these phenomena are dependent upon the position of the cell within the lobule and the consequent nature of the blood supplying the cell.

SUMMARY AND CONCLUSIONS

1. The livers of a series of mice from birth to 45 days of age have been studied.

2. Glycogen is present in the hepatic cells at the time of birth and rises in amount until the eighteenth day.

3. Although a large amount of fat is accumulated in the liver at the time of birth it disappears rapidly, first from the center of the lobule.

4. The secretion of bile acids appears to begin at the end of the first week of postnatal life and increases with age.

5. Antagonisms do not exist between the storage of glycogen and fat nor between the storage of glycogen and the secretion of bile acids.

6. There is no variation in the form, size, and staining reaction of the mitochondria within the lobule at birth, but a zonation in these develops with age.

7. The Golgi substance exists first as granules lying peripherally in the cell; these later fuse into networks that lie both juxtanuclearly and peripherally.

8. Again there are no conclusive indications of direct relationships between changes in the cell organelles and in the processes of storage and secretion studied. All zonation phenomena are most probably dependent upon the nature of the blood supply of the liver lobule.

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* Read only in abstract.

EXPLANATION OF PHOTOMICROGRAPHS

The photographs were taken using apochromat objectives and compensating oculars. Figures 9 and 10 were taken with a 16-mm. (10 ×) objective, the rest with a 2-mm. oil immersion (90 ×) objective. The pictures of Heidenhain iron-alum hematoxylin preparations (for mitochondria) and of Champy-Kolatchev preparations (for the Golgi substance) were taken without any filter. Those of Forsgren preparations (for bile acids) were taken with a Wratten blue filter. Those of Bauer-Feulgen preparations (for glycogen) and of sudan IV preparations (for fat) were taken with a Wratten X_1 green filter.

PLATE 1

EXPLANATION OF FIGURES

1 3-day ♂. Regaud-Heidenhain preparation. Central zone. Mitochondria are heavy rods. Cell undergoing chondriolysis. Magnification, × 1350.

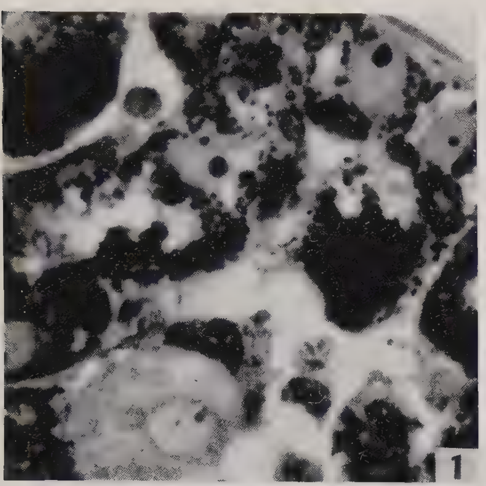
2 13-day ♂. Regaud-Heidenhain preparation. Peripheral zone. Mitochondria are heavy spheres. Magnification, × 1350.

3 24-day ♀. Regaud-Heidenhain preparation. Central zone. Mitochondria long, light filaments. Magnification, × 1350.

4 45-day ♂. Regaud-Heidenhain preparation. Peripheral zone. Mitochondria are short, heavy rods. Magnification, × 1350.

5 9-day ♀. Champy-Kolatchev preparation. Golgi substance consists of granules near nuclei, in the intermediate cytoplasm, and along the bile capillaries. Magnification, × 1350.

6 19-day ♀. Champy-Kolatchev preparation. Golgi substance consists of small, tight nets near the nuclei and adjacent to the bile capillaries. Magnification, × 1350.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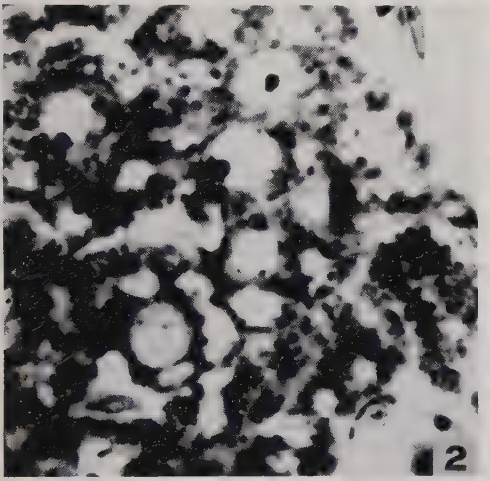
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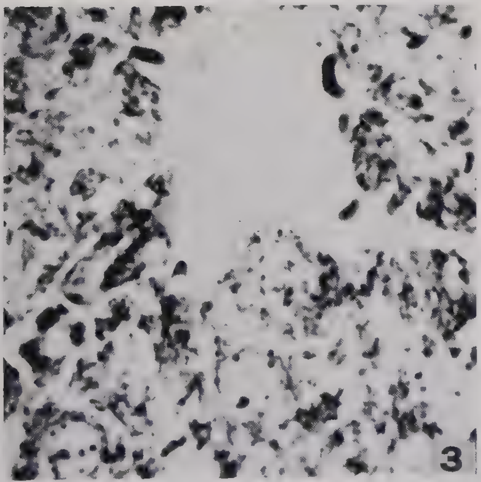
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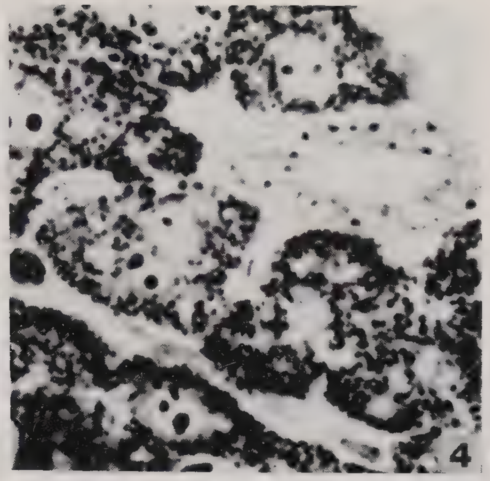
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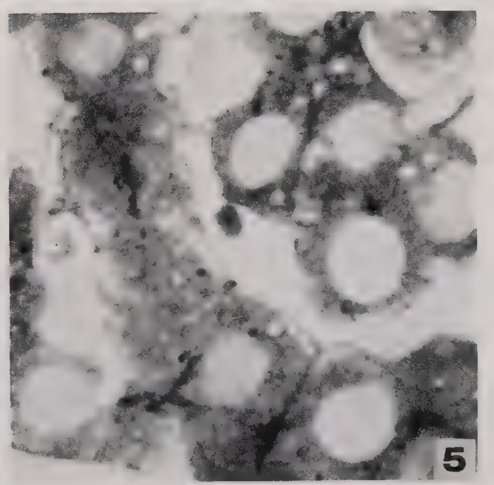
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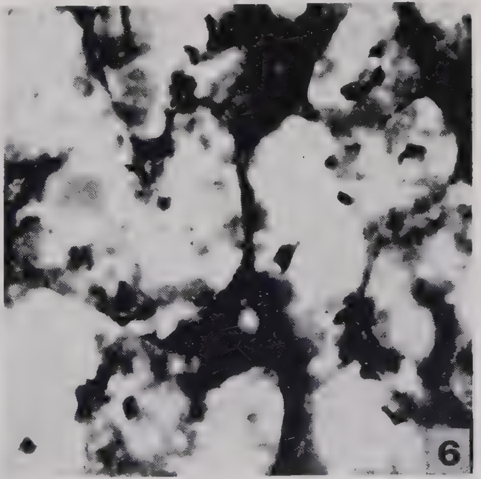
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PLATE 2

EXPLANATION OF FIGURES

7 45-day ♂. Champy-Kolatchev preparation. Golgi substance consists of fragmented networks lying equatorially across cells. Magnification, × 1350.

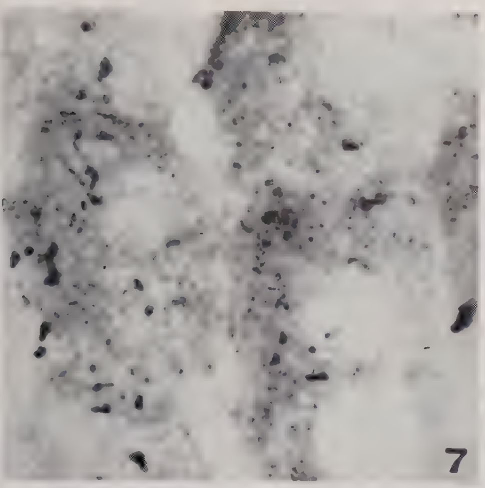
8 9-day ♂. Forsgren preparation. Middle zone. Bile acid granules small and very crowded. Magnification, × 1350.

9 9-day ♂. Bauer-Feulgen preparation. A small amount of glycogen is present in all of the hepatic cells, more in some. Magnification, × 110.

10 19-day ♀. Bauer-Feulgen preparation. Good deal of glycogen in all cells. Magnification, × 110.

11 1-day ♀. Sudan IV preparation. Many large droplets of fat in hepatic cells. Magnification, × 566.

12 13-day ♀. Sudan IV preparation. Liver cells contain fewer fat droplets. Note persisting hemopoietic tissue. Magnification, × 760.



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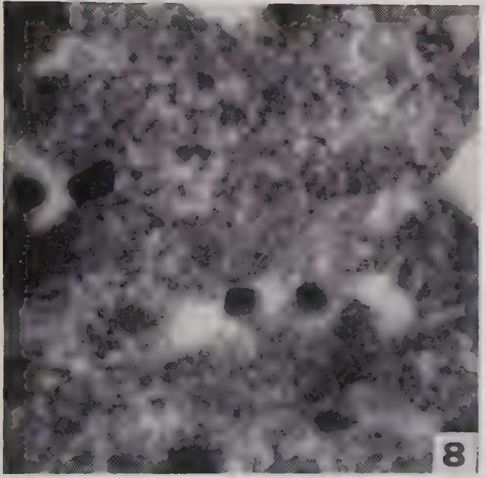

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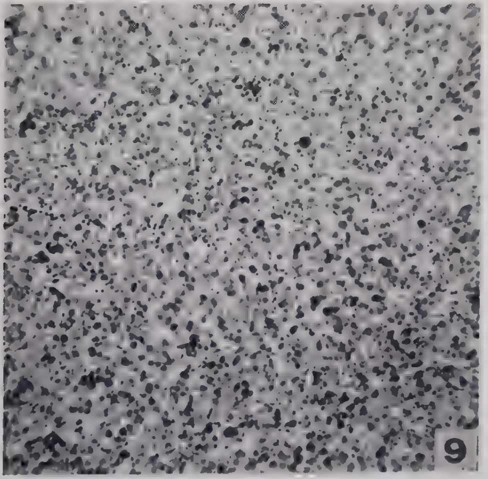

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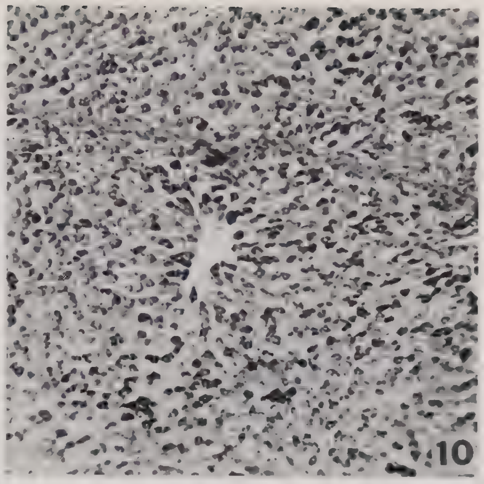

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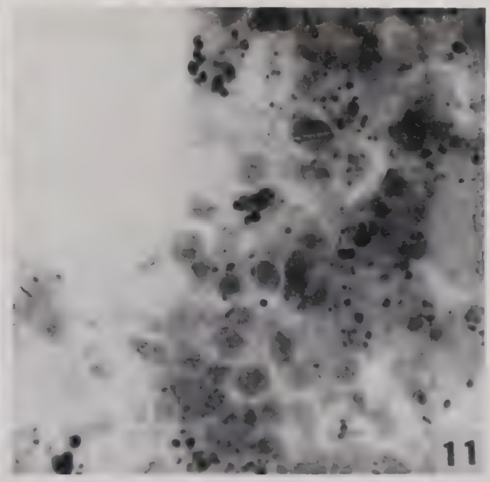

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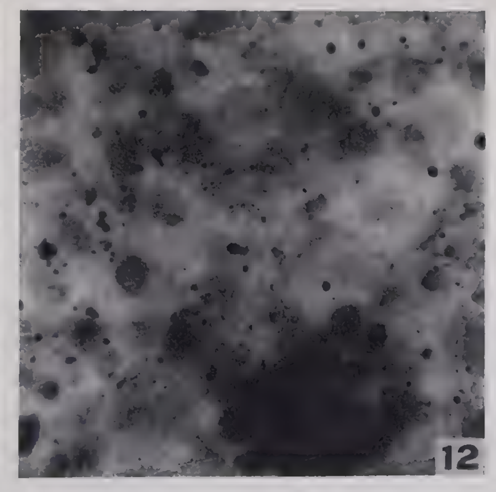

12

THE DRAINAGE OF PARTICULATE MATTER FROM THE PERITONEAL CAVITY BY LYMPHATICS

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ELEVEN FIGURES

INTRODUCTION

When particulate matter is injected into the peritoneal cavity of a cat, particles become adherent to the diaphragm, greater and lesser omenta, and to other areas of the peritoneum. Those granules attached to the diaphragm rapidly enter the lymph vessels of that organ and pass by way of parasternal trunks to sternal and mediastinal lymph nodes. A large part of the material remaining in the peritoneal cavity adheres to the greater omentum and is thereby almost completely immobilized, apparently by means of a fibrinous deposit covering the mesothelial cells. A few of the adherent granules may enter these cells and remain in them for some time, but most of the particulate matter is picked up by phagocytes and carried to the deeper interstitial connective tissue of the omentum. The supposition has been that the granules, or part of them, ultimately find their way into the general circulation by entering omental blood or lymph capillaries. The lymphatic route has been mentioned but not emphasized by previous investigators, for the existence of omental lymph vessels has been questioned. Since it is now established that these vessels do exist in profusion and are tributaries of definite lymph nodes, the primary purpose of this study was to examine the omental lymphatics and their associated nodes to discover the part they play in removing particulate matter from the peritoneal cavity. Observations on the thoracic lymph vessels and nodes also engaged in this cavity are included in this report.

LITERATURE

Numerous studies have been made of the many problems associated with peritoneal absorption. Cunningham ('26) gives an excellent discussion of this field and reviews the literature pertaining to it. A concise summary of those articles dealing specifically with the absorption of particulate matter is presented by Mackmull and Michels ('32).

Only the more recent papers, and those dealing directly with the problems at hand, are considered in this review.

A most perplexing question associated with absorption of particulate matter from the peritoneal cavity involves the method by which particles pass into diaphragmatic lymph vessels. Peritoneal stomata, described by von Recklinghausen (1863) and generally accepted for many years, provided a satisfactory answer to this problem. These structures were later interpreted as artefacts, and the existence of stomata has been denied by a majority of investigators since the adverse findings of Hertzler ('01) and MacCallum ('03). Recently, Allen ('36), reopened this discussion with a study of the mouse diaphragm in which he describes normal and physiological openings, peritoneal stomata, overlying lymphatic lacunae. His interpretation of these openings differs from the original description of stomata, for he considers them comparable to tissue spaces from which particulate matter enters lymph vessels by passing between lymphatic endothelial cells when such cells are pulled apart temporarily during expiration.

The importance of the lymphatic pathway from the diaphragm along sternal vessels to mediastinal nodes has been noted by Muscatello (1895), Durham (1897), Buxton and Torrey ('06), Bolton ('21), Cunningham ('22) and Brown ('28). Higgins and Bain ('30) estimate that in the dog, 80% of the graphite injected into the peritoneal cavity is removed along this pathway. Buxton and Torrey record that ink, injected into the peritoneal cavity of a guinea pig, appears in the parasternal trunks within 15 minutes. At first the ink lies free in the lumina of the vessels and the peripheral sinuses of the mediastinal nodes, but soon many gorged macrophages are evident among the free particles. Later, all ink observed in the nodes was phagocytized. These observations were continued for 2 months after injection, and to date no one has reported studies covering a longer post-operative period.

Heger ('04) was among the first to emphasize that the omentum also provides a pathway for the removal of particulate matter from the abdominal cavity. Although Buxton and Torrey recognized the importance of this organ, they were primarily concerned with the manner in which particles are held in contact with the mesothelial surface, and later phagocytized. Shipley and Cunningham ('16) and Poynter ('28) state that free particles are taken into omental veins but the prevailing opinion has always favored lymphatics as an avenue of transportation. Buxton and Torrey claimed to have found lamp black, previously injected into the peritoneal cavity, in the afferent plexuses of the lymph nodes of the omentum, but Cunningham ('26) questioned this observation on the basis that no other investigator recognizes such nodes. This criticism is not justified, for Baum ('18) described an omental node in the dog and considers efferent channels from this node as well as others from the omentum as important tributaries to the duodenal node. Recent studies by the author ('34, '35), Dick ('35), and Webb and Simer ('42) leave no doubt as to the presence of omental lymph vessels and collecting nodes in common laboratory animals and man.

MATERIALS AND METHODS

Twenty-two young adult cats were used in this study. Lean animals were selected to avoid excess fat accumulations in the omenta. Five cats were given ether anesthesia, after which midline abdominal incisions were made. A plug of gauze was dampened in dilute india ink or a 1 to 60 indigo carmine solution and rolled in the omentum. The incisions were closed by temporary sutures for periods of 1½ to 5½ hours, after which animals were killed. Post mortem examinations included gross and microscopic study of the thoracic and abdominal lymph nodes and vessels, with special emphasis on those associated with the omentum.

The remaining seventeen animals were injected with 2 cc. of a suspension consisting of equal parts of india ink and normal saline solution. The first injections were made by forcing the needle through the abdominal wall near the midline, using care to avoid injury to internal organs. Later examinations proved that in some instances such injury had occurred, for ink blotches were observed in the intestinal wall, urinary bladder or anterior abdominal musculature. To avoid this difficulty ten of the cats were treated in a different manner. Each was anesthetized and a short midline incision was made in the skin and superficial fascia of the anterior abdominal wall. The hypodermic needle was then forced through a retracted portion of the muscle, without injury to other structures. These animals were sacrificed and routine post mortem examination made at intervals from 12 hours to almost 18 months after operation.

OBSERVATIONS

Thorax. The thoracic lymph nodes of the cat have the same general arrangement as in the dog, as described by Baum ('18). No intercostal nodes were observed in any specimens but they take no part in removing ink from the peritoneal cavity and small ones may have been overlooked. Two or more nodes compose the sternal group, lying immediately posterior to the sternum at the level of the second costal cartilage. Their principal tributaries are the parasternal lymph trunks which, in turn, drain the lymph vessels of the diaphragm. A cranial mediastinal group of nodes is quite constant, but the number, size, and distribution of the individual members of the group show much variation. One large node is located in the angle between the right and left carotid arteries and additional ones lie at about the same level, associated with the right and left subclavian arteries. All mediastinal nodes receive tributaries from the sternal group. Bronchial nodes, situated along the bronchi and at the bifurcation of the trachea, were observed in all animals where special search was made. They and the adjacent pulmonary nodes are small and are well hidden in the surrounding tissues.

In those animals where a plug of gauze was dampened with india ink or indigo carmine solution and rolled in the tail of the omentum in such a manner that no ink came in contact with the diaphragm, no thoracic lymphatics appeared colored at gross examination. But in every instance where free ink was injected into the peritoneal cavity, many lymph vessels and nodes of the thorax were intensely colored. Within 15 minutes the subpleural lymphatics of the diaphragm are so filled that their general pattern is beautifully delineated. These vessels are parallel to the muscle fascicles, and lie in shallow furrows between them, extending radially from the periphery of the central tendon. Transverse collecting vessels unite the central and peripheral ends of the radial radicles and conduct most of the lymph from the diaphragm toward the xiphoid process of the sternum. In this way, two large parasternal trunks are formed, one on either side, near the lateral borders of the sternum. These sternal trunks drain the diaphragm and receive other afferent vessels from the intercostal spaces. They constitute the most important afferent lymphatics to the sternal nodes.

For 2 or 3 days after injection, the sternal trunks remain jet black and markedly inflated, and the same conditions obtain in the nodes. At the end of this time the vessels became less distinct, were often interrupted, and in a few cases the color faded so they could not be identified on both sides of the sternum. Eighteen months after operation, faintly colored, segmented, gray, substernal lines could be seen, leading to the heavily pigmented sternal lymph nodes. The mediastinal group of lymph nodes never shows such general and massive accumulation of ink. Some of them were slightly colored at the second day after operation, but it was rare for all to be pigmented. No ink was observed grossly in any nodes of this group later than 8 months after operation.

A study of microscopic sections of thoracic lymphatics from 1 hour to 2 days following peritoneal ink injection revealed an abundance of free particles in the lumina of the subpleural vessels of the diaphragm, the parasternal lymph trunks (fig. 1), and in the peripheral sinuses of sternal nodes (fig. 2). This finding is in accord with the report of Buxton and Torrey ('06) and suggests the probability of actual openings in the peritoneum covering the diaphragm, through which particulate matter may pass directly into the lymph vessels of that organ. The speed with which free ink appears in thoracic lymphatics supports this assumption. The observations of Allen ('36) are of interest here, for he contends that normal and physiological openings actually are present in the mouse diaphragm, permitting particles to pass directly from the peritoneal cavity into subperitoneal lymphatic vessels. No attempt was made in this investigation to repeat Allen's experiments, but it may be recorded that no actual peritoneal openings were observed in micro-

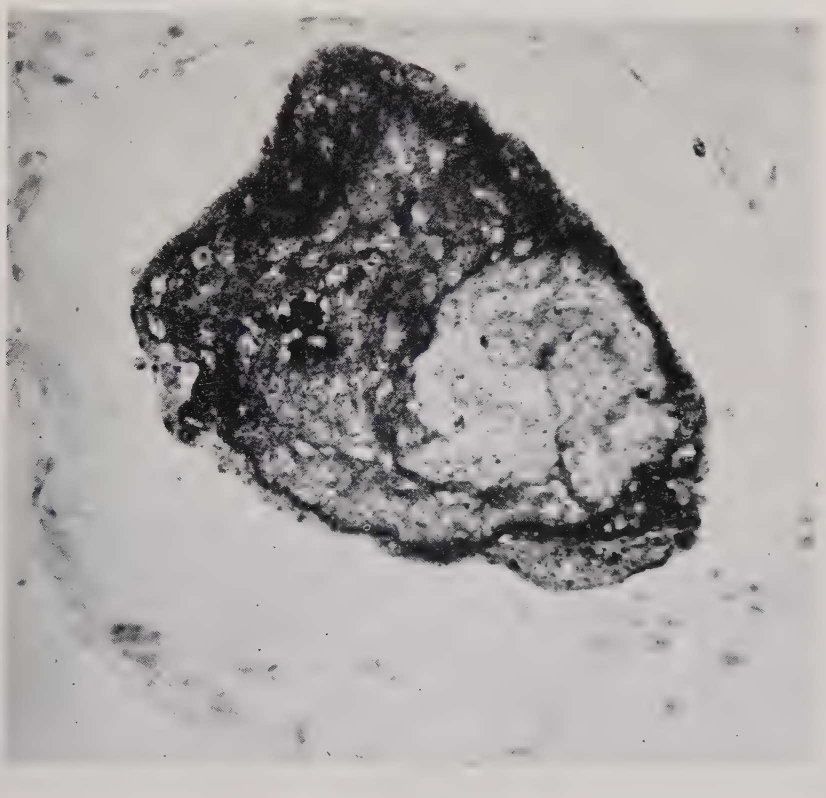

Fig. 1 Photomicrograph of a section of a parasternal lymph trunk 5½ hours after intraperitoneal injection of ink. Note preponderance of free ink granules in the lumen. × 360.

scopic sections of the cat diaphragm. The two surfaces of this organ show marked differences, for the pleural side is smooth and contains a thick layer of connective tissue between the parietal pleura and muscle, while the opposite side is uneven with a small amount of extramuscular connective tissue (fig. 3). There are many areas on the abdominal surface where the subperitoneal lymph vessels are separated from the peritoneal cavity only by narrow septa. The septa are not uniform in thickness and often contain one or more shallow pits that may well be potential openings (fig. 4). No ink particles were observed passing through the septa or lodged in the pits, but the numerous granules on both sides of the partitions suggest that particulate matter may pass through them into the lymph vessels of the diaphragm.

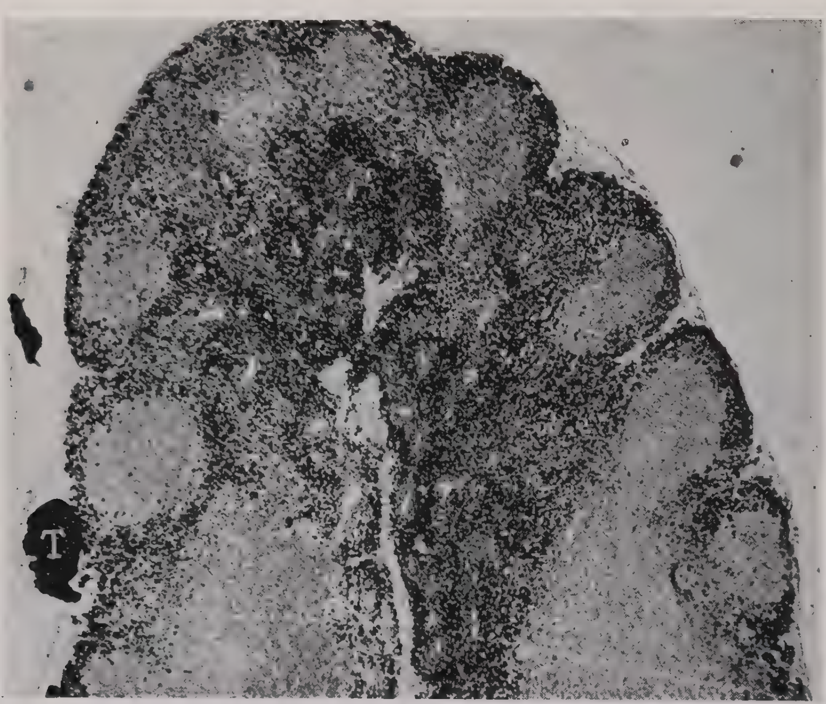

Fig. 2 Photomicrograph of a section of the sternal lymph node 12 hours after intraperitoneal ink injection. The termination of the parasternal trunk, T, and the peripheral and cortical sinuses of the nodes are crowded with free ink particles. Some phagocytes are seen in the node. × 50.

Microscopic sections of sternal lymph trunks and nodes 1 day after peritoneal injection of ink show many gorged phagocytes surrounded by an abundance of free ink particles. In the trunks, the phagocytes show a tendency to be clumped along the peripheral walls or those of a valve cusp, while in the nodes, both phagocytes and free ink have crowded into the septal sinuses and the medulla (fig. 5). Two days after injection, almost all free ink has been phagocytized (fig. 6). There is little doubt that macrophages are of great importance in this process, but the endothelial cells of the lymph trunks cannot be ignored. They contain numerous granules, a few in each cell, which show distinctly in transverse sections of the large lymph vessels (fig. 7).

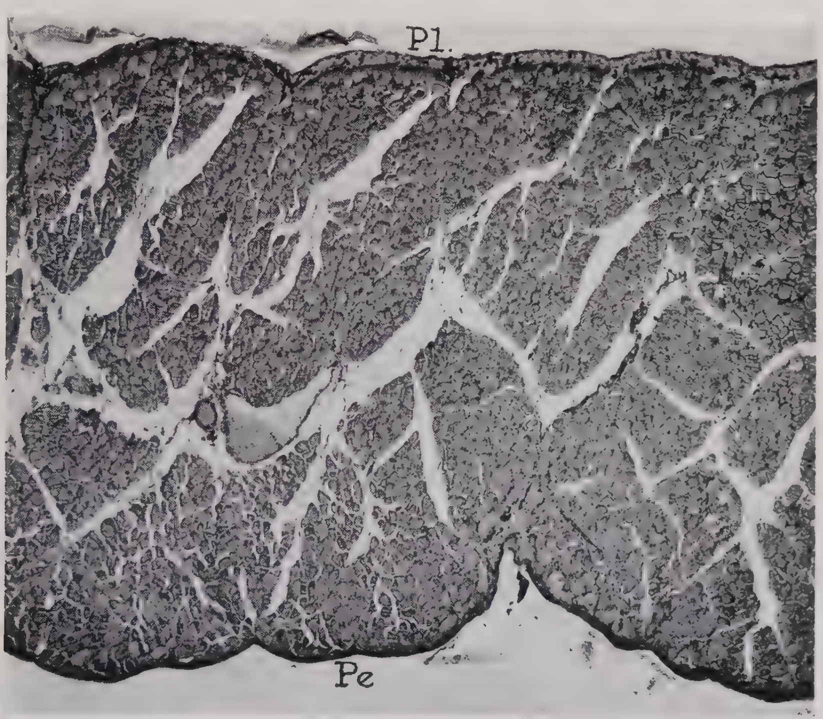

Fig. 3 Photomicrograph of a section of cat diaphragm. Note difference in pleural, Pl, and peritoneal, Pe, surfaces. × 50.

A remarkable change has occurred by the end of the third day after operation. At this time the lymph stream has swept the phagocytes from sternal trunks into collecting nodes, leaving the vessels free of ink. Each trunk is embedded in connective tissue, composed largely of fat cells and connective tissue fibers. This tissue now contains large numbers of ink-filled phagocytes, some of which crowd against the lymph and blood vessels (fig. 8). It is this accumulation that gives the black color to the area of the parasternal trunks, so noticeable in gross examinations. This unexplained condition persists for an extended time and is still evident almost 18 months after ink injection. The perivascu-

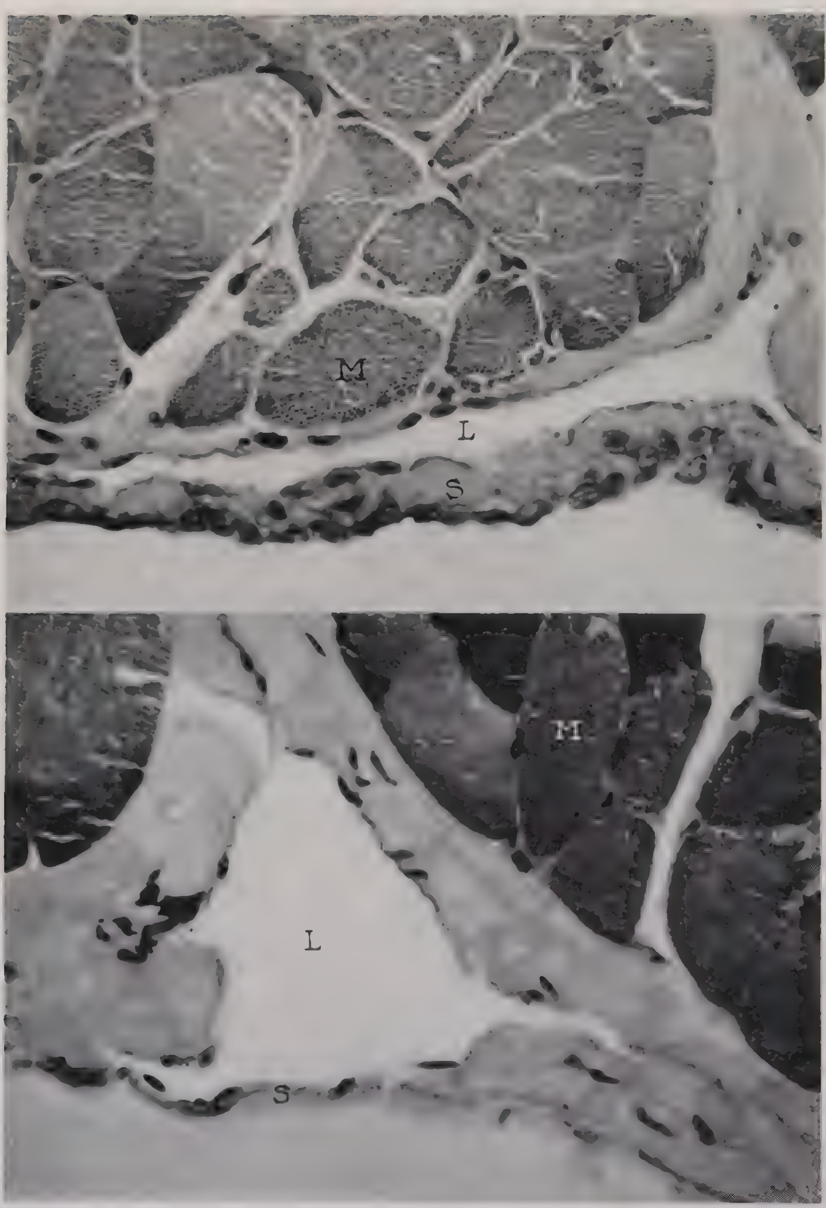

Fig. 4 Photomicrographs of sections of cat diaphragm, showing subperitoneal lymph vessels and the narrow septa separating them from the peritoneal cavity. S, septum; L, lymph vessel; M, striated muscle. × 520.

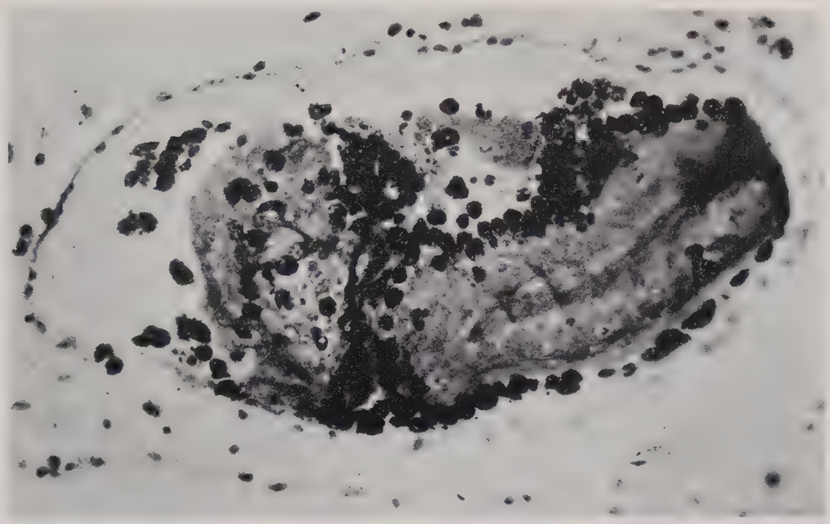

Fig. 5 Photomicrograph of a section of parasternal lymph trunk 24 hours after injection. Note abundance of phagocytes. Compare with figure 1. × 280.

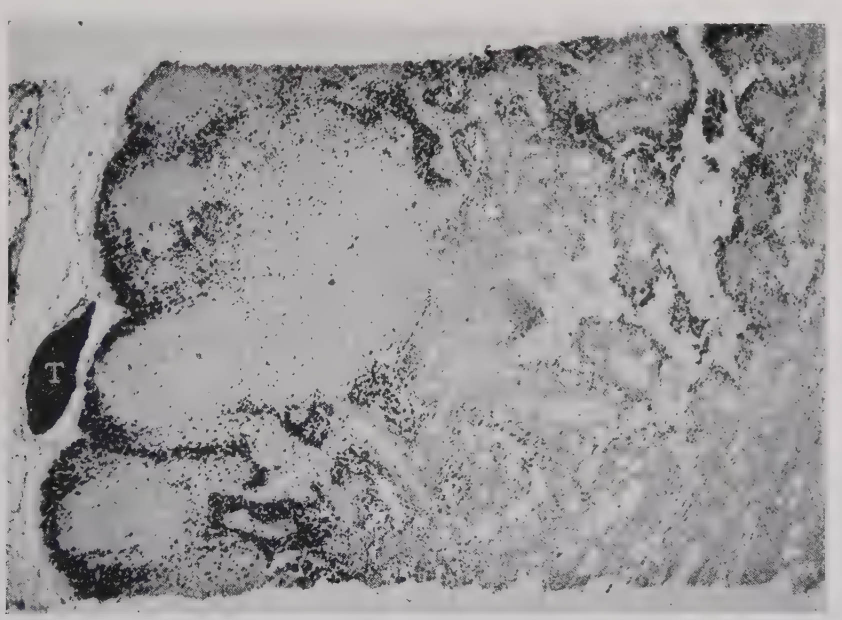

Fig. 6 Photomicrograph of a section of sternal lymph node 48 hours after ink injection. Most of the ink in the node has been phagocytized, but the terminal portion of the parasternal trunk, T, is filled with free ink particles. × 43.

lar tissue thus serves as a storehouse for particulate matter, much as do the lymph nodes to which the subpleural and parasternal vessels drain.

Abdomen. The abdominal lymph nodes in the cat have the same general location as in the dog. The nodes which receive lymph from the omentum are the duodenal, splenic, and portal, but of these, only the first two represent primary collecting points. The omental node, present in many dogs, was not observed in any cats in this study. Efferent lymph vessels from the right three-fourths of the omentum lead directly into the duodenal node, situated ventral to the pyloric-duodenal junction. The remaining lymph drainage from this organ is to the small splenic nodes, usually two or three in number, lying near the splenic vein. The efferent vessels from both duodenal and splenic nodes lead to the portal group, consisting usually of one on either side of the portal vein. Gastric lymph nodes are located in the lesser omentum at the lesser curvature of the stomach, and along the short gastric veins dorsal to this organ. They do not receive lymph from the omentum, but were always colored when any portion of the stomach wall was accidentally punctured by the injecting needle.

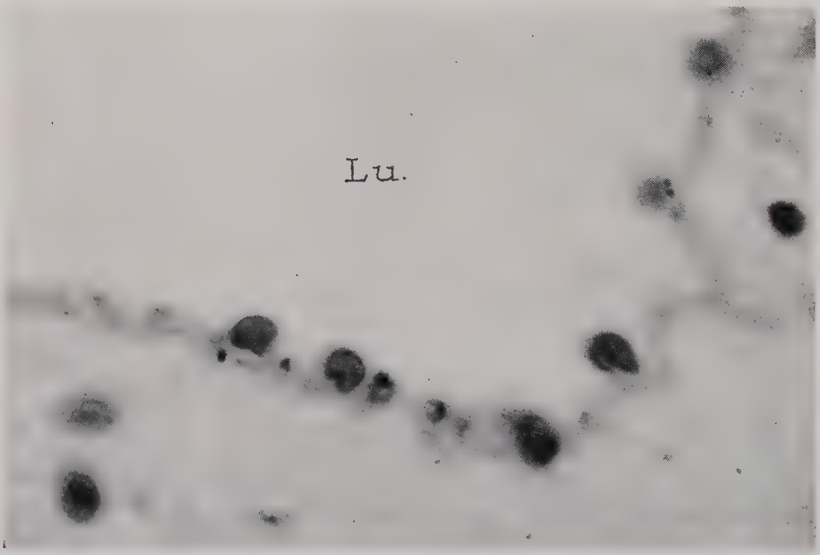

Fig. 7 Photomicrograph of a section of a parasternal lymph trunk, 3 days after ink injection, showing a portion of the lumen, Lu. The endothelial cells contain ink granules. × 1170.

In addition to those nodes listed, there are others which were observed in routine post mortem examinations. These include the jejunal and

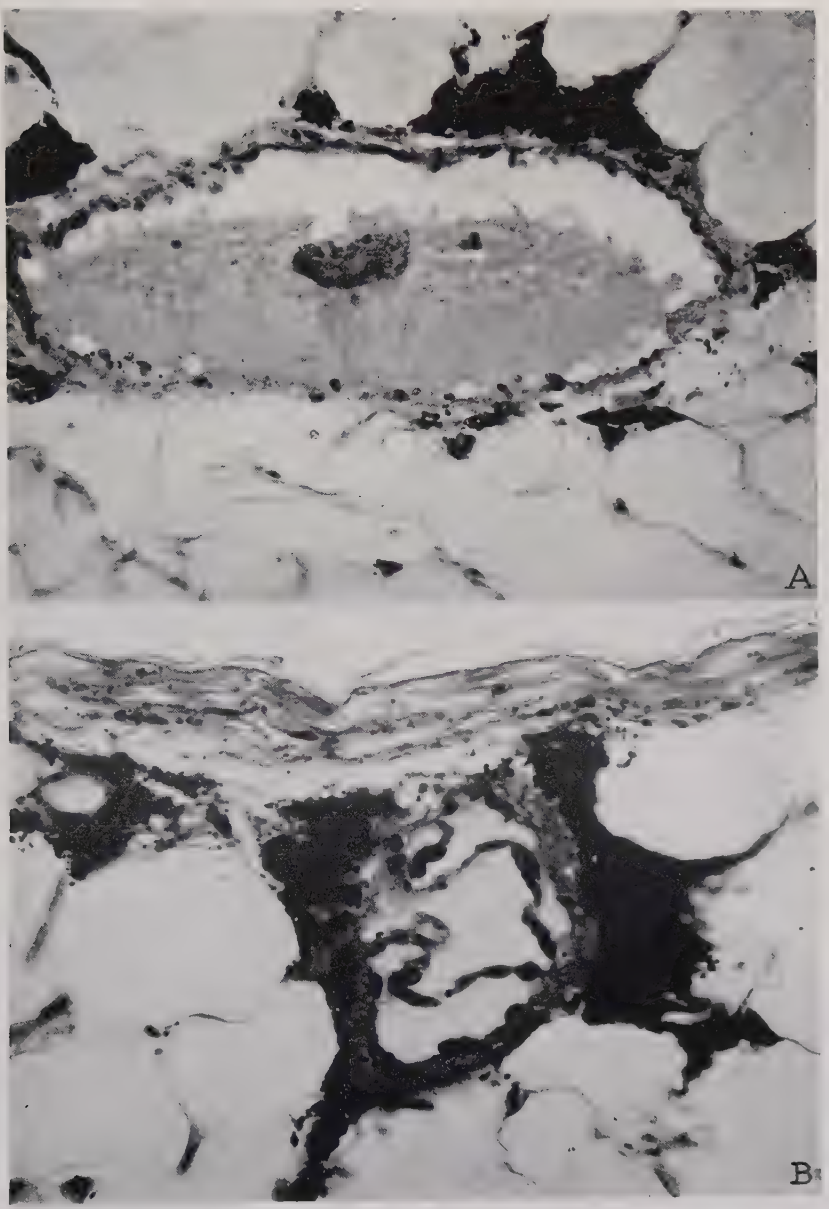

Fig. 8 Photomicrograph of sections of parasternal lymph trunks. A, 36 days after ink injection. × 600. B, 6 months after ink injection, × 325. Note dense accumulations of ink outside lymph vessels and only a few granules inside them.

colonic groups, associated with corresponding portions of the intestine, and the median iliac, hypogastric and sacrals, located at the bifurcation of the abdominal aorta and at the origin of its subsequent branches. There was no evidence that these nodes play any part in removal of particulate matter from the peritoneal cavity, for none showed pigmentation at gross examinations except in those cases where the body wall and viscera were injured at injection. A cranial pair of lumbar nodes, however, was always intensely black within a few minutes after ink was introduced into the peritoneal cavity. These nodes lie on either side of the aorta, cranial to the origin of the renal arteries, and receive afferent vesssls from the dorsal portion of the diaphragm, a fact noted also by Higgins and Bain ('30). The speed with which ink from the peritoneal cavity reaches them is further proof of the rapid flow of particulate matter from that cavity into the lymph vessels of the diaphragm.

The dense accumulation of particulate matter on the omentum immediately after peritoneal injection is an observation made by all investigators in this specific field. Profuse omental lymph vessels have been described in previous articles by the author ('34, '34a, '35), by Dick ('35) and by Webb and Simer ('42). The proximity of the lymph vessels and particles would seem to provide ample opportunity for drainage of particulate matter by way of omental lymphatics, comparable if not equal to that occurring in the diaphragm. Since the duodenal node receives the major portion of the lymph from the omentum, it acts as the primary collecting point for any particulate matter transported by omental lymphatics and thus serves as a reliable index of the functional activity of these lymph vessels. It bears the same functional relationship to lymph vessels in the omentum that the sternal nodes do to lymphatics in the diaphragm.

The observations of Braithwaite ('23) indicate that omental lymphatics are of importance in removing certain substances from the peritoneal cavity. He saturated small pieces of cloth with indigo carmine solution (1 to 60) and attached them to the omenta of adult patients at laparotomy. One and one-half hours later, in four of twenty-one cases, he was able to trace omental lymph vessels from the cloth to the right gastroepiploic nodes by means of absorbed color. My attempts to secure similar results in three cats were complete failures, and when an india ink suspension was substituted for the indigo carmine solution there was no indication that granules of carbon had entered the lymph vessels or nodes. A study of numerous microscopic sections of the omenta and duodenal nodes showed no instance of granule accumulation in the lymphatics. It has been shown, however, that these vessels do absorb certain dye solutions and transport them to adjacent lymph nodes. When a 1% aqueous solution of trypan blue is injected into the peritoneal cavities of rabbits and cats, the larger omental lymphatics stain so intensely that their pattern may be observed grossly. All lymph nodes receiving tributaries from the omentum are colored blue (Webb and Simer, '42).

Gross examinations of cat omenta from 1 hour to 18 months after intraperitoneal ink injections show that particulate matter does not enter and delineate the pattern of the lymph vessels of this organ. Adherent and phagocytized particles are retained by the mesothelial cells or are transferred to the connective tissue between fat cells and surrounding blood vessels. This ink deposit remains intact for a remarkably long period, for the color of the omentum is essentially the same 18 months after peritoneal injection as it is 1 day after the introduction of particulate matter. It is characteristic that long after ink particles have disappeared, grossly, from the preperitoneal fat, mesentery, and recto-uterine folds, the omentum alone retains its dark color (fig. 9).

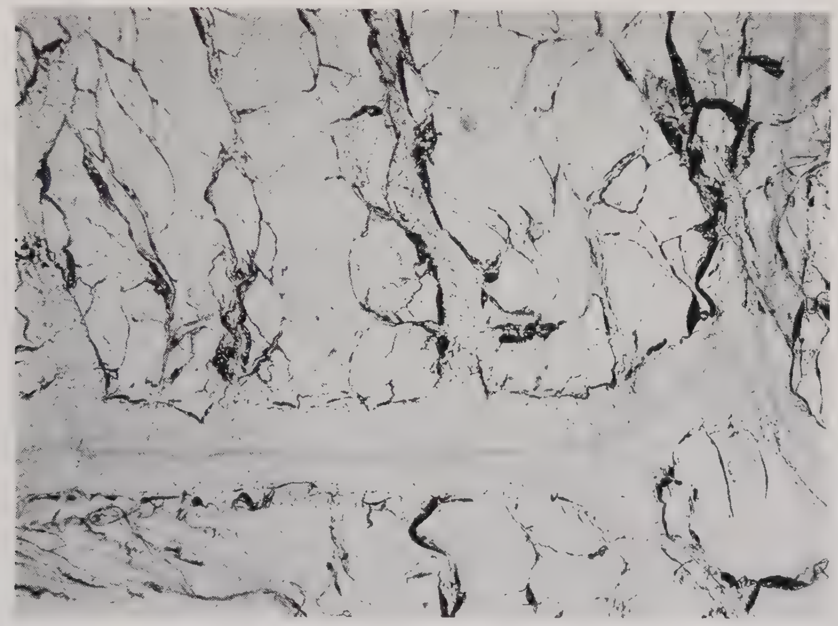

Fig. 9 Photomicrograph of a portion of an omental spread, 10 months after ink injection. Note areas of ink accumulation. This picture is not materially changed 8 months later. × 5.

Careful study of serial sections from numerous areas of the omentum show that some ink does enter the omental lymphatics. Occasionally one finds a few phagocytes lying in the lumina or near the walls of the larger lymphatics and some of them contain ink granules (fig. 10). These cells are never numerous in any one area, and seldom contain more than a few granules in each cell. It is a matter of considerable interest that the phagocytes are usually found in the larger, more peripheral lymph vessels of a fat strip, and not in the small channels which form the central perivascular plexus. Since no phagocytes or ink particles were seen entering lymphatics from the peritoneal cavity, it is not possible to state exactly how this transfer is accomplished. But it is worthy of note that restricted segments of the larger lymph vessels often lie in a peripheral, submesothelial position in a fat strip, then dip deeper into the tissue and continue in their course. In such exposed areas, the lumen of the lymphatic is separated from the peritoneal cavity by a narrow septum, consisting of the endothelial lining of the vessel and the mesothelium covering the omentum. These narrow bridges may serve as the avenue for entrance of ink particles, and the almost complete absence of phagocytized particles in omental vessels as contrasted with diaphragmatic channels and parasternal trunks may be correlated with the relatively small area of exposed omental lymphatics. It is quite improbable that any great number of these

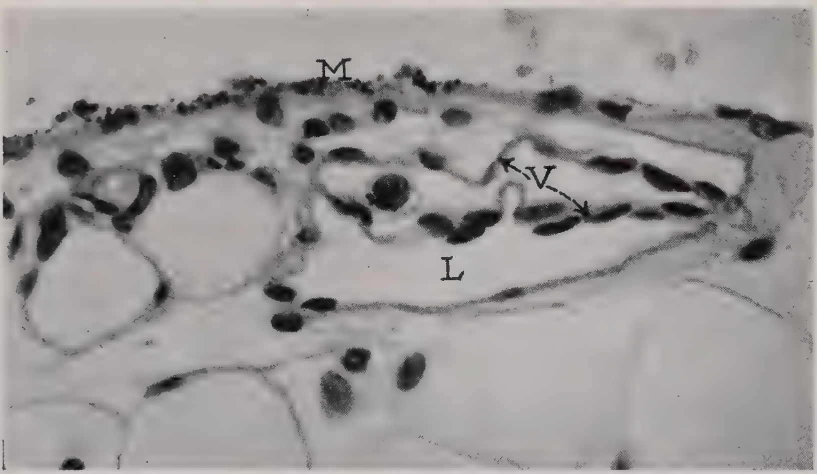

Fig. 10 Photomicrograph of a section of an omental fat strip, 24 hours after injection, containing a lymph vessel near the mesothelial surface. Note a phagocyte containing several ink granules in the lumen of the vessel, between valve cusps. L, lymph vessel; V, valve cusp; M, mesothelial cells with adherent and ingested ink granules. × 750.

phagoctyes which are lodged in the interstitial tissue of the omentum ever get into the lymph stream, for no increase of cells was noted in the vessels during the months following injection, the color of the omentum does not change materially, and, as will be noted later, the duodenal node does not show marked additional coloration.

The position and afferent lymph supply of the duodenal node make it the most important primary collecting point for the lymph flow from the omentum. Any colored solution or particles passing from the peritoneal cavity into the omental lymphatics soon reach this node and impart a distinct color to it. Gross and microscopic studies of the node support the conclusion that the lymph vessels of the omentum play a minor role in removing particulate matter from the peritoneal cavity. It is almost 12 hours after ink injection before this node shows a light stippling of pigment, and then only at the caudal pole. The density of color may increase slightly during the next few days, but it never becomes intense, and never involves more than a small fraction of the entire node. Microscopic sections taken 18 months after operation show only a few ink granules distributed widely in the medulla at the caudal pole, and thus indicate that a relatively small amount of particulate matter passes through the omental lymphatics during this time (fig. 11).

Splenic lymph nodes show about the same degree of activity as noted in the duodenal node. Color was first observed in them 1 day after ink injection, and in animals sacrificed at longer postoperative periods, a slight amount of pigment was always noted. The splenic nodes are quite small and variable in position, often being completely hidden in fat accumulations. This, coupled with the fact that they receive only a small number of omental lymph vessels, makes them an unreliable index of the activity of the lymphatics of that organ. The portal nodes were equally unsatisfactory as a source of information in this study. They are often difficult to locate and show color relationships very poorly. Since they are secondary collecting centers of omental lymph, they contain pigment only after it has appeared in duodenal and splenic nodes. The observations made in gross and microscopic studies of portal nodes were of little value except that they served as confirming evidence.

It is evident from this study that the omentum immobilizes and retains in its connective tissue a large part of particulate matter injected into the peritoneal cavity. The areas of retention are the connective tissue between fat cells, the perivascular connective tissue, the milk spots, and the mesothelial cells covering the organ. But the omental

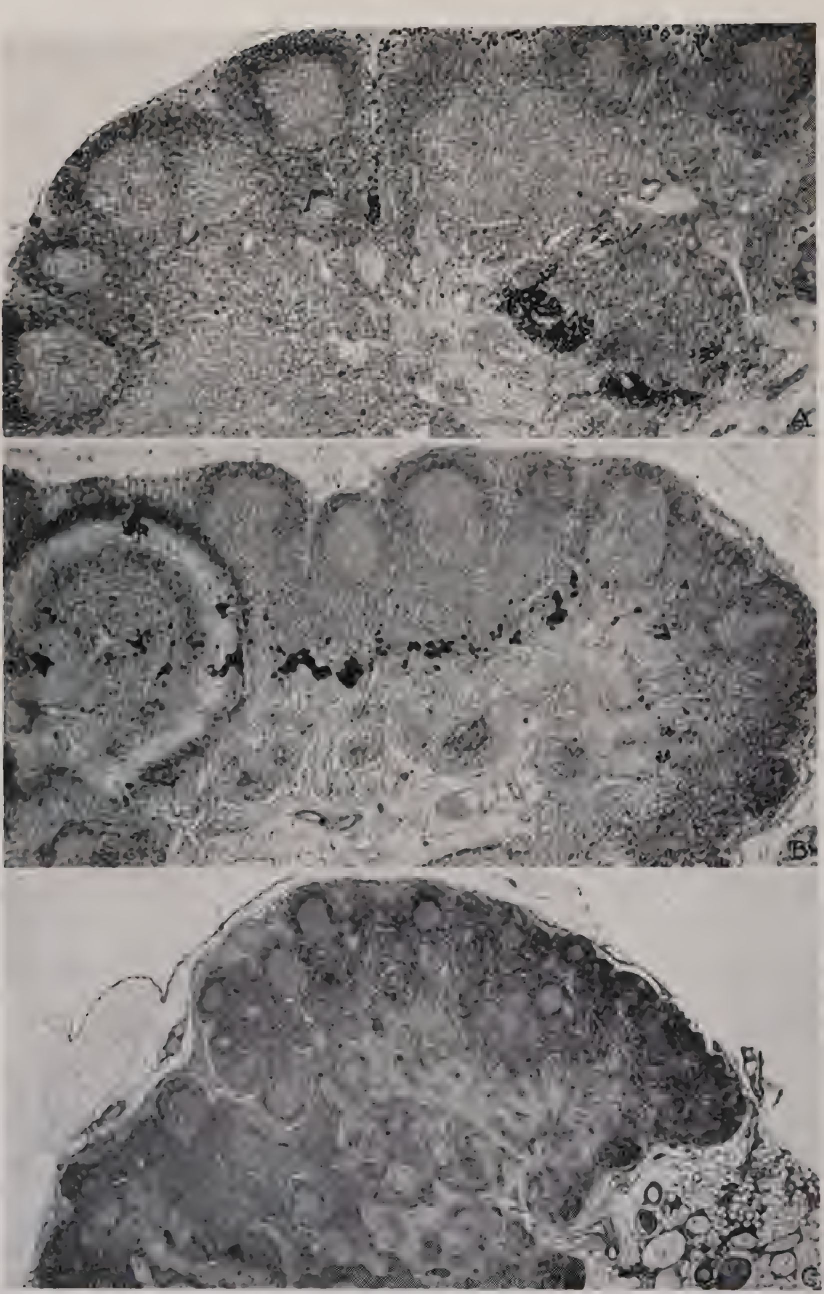

Fig. 11 Photomicrographs of sections of duodenal lymph nodes of the cat. A, 12 hours after intraperitoneal ink injection. × 55. B, 2 months after injection. × 40. C, 18 months after injection, × 25. Note differences in the location and amount of ink in these three sections.

lymphatics and associated nodes, quite in contrast with those of the diaphragm, play little part in removing free particles from the peritoneal cavity or from the connective tissue of the omentum itself.

SUMMARY

1. India ink or indigo carmine solution was placed in the peritoneal cavities of twenty-two cats. In seventeen of the animals the ink was injected through the abdominal wall and in five others, a gauze sponge, saturated with ink or indigo carmine solution, was wrapped in the tail of the omentum at laparotomy.

2. Animals were killed at periods varying from $1\frac{1}{2}$ hours to $1\frac{1}{2}$ years after injection. Gross and microscopic studies were made of those thoracic and abdominal lymphatics which showed evidence of taking up particulate matter.

3. The lymph vessels of the diaphragm contained free ink within a few minutes after injection. The same is true of the parasternal lymph trunks and the sternal lymph nodes. After the first day, large ink-filled phagoctyes increased in number among the free particles. By the end of 1 week, all lymph vessels were clear, but their location was indicated grossly by an accumulation of ink-filled phagocytes in the surrounding connective tissue. This tissue and the sternal and mediaastinal lymph nodes retained the stored pigment during the entire 18-month period that animals were observed.

4. In the abdomen, the omentum is the most prominent area of ink adhesion. This organ was immediately blackened by adherent particles, and retained the pigment during the entire course of this investigation. Some of the particles enter the mesothelial covering cells, but most of them are phagocytized and stored in the connective tissue. Although there is a rich plexus of lymph vessels in the omentum, few ink-filled phagocytes or free particles were found within these channels. Most of the lymph vessels of this organ empty into the prominent duodenal node, but a few drain to the small splenic nodes. The duodenal node contains ink on gross examination 12 hours after operation, but only in small amounts, at the caudal pole. It never contains large accumulations of pigment, spread throughout the node. Splenic nodes contained ink 1 day after injection, but they were often only lightly stippled with the color.

5. The paucity of particulate matter in omental lymph vessels and duodenal lymph nodes following injection of india ink into the peritoneal cavity indicates that omental lymphatics are of very little importance in removing particulate matter from this cavity.

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FURTHER STUDIES ON THE SURVIVAL OF SPERMATOZOA IN THE FEMALE REPRODUCTIVE TRACT OF THE BAT[1]

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The present paper describes further experiments undertaken to determine, conclusively, whether spermatozoa introduced during the fall into the female reproductive passages of the bat are able to survive throughout the several months of hibernation and fertilize the ova that are discharged from the ovary upon the termination of hibernation in the spring. In a previous publication (Wimsatt, '42), conception was described in two female bats (Eptesicus) which had been isolated from males for 140 and 150 days respectively. The experiments now to be reported extend the observations made in the earlier paper to a larger series of animals, and, in addition, observations are included for another genus of bats (Myotis).

Inasmuch as Hartman ('33), Baker and Bird ('36), and Wimsatt ('42) have thoroughly reviewed the history of the problem of the survival of spermatozoa in the hibernating female bat, there is no need to present again a detailed statement. In general, the observations of workers previous to 1933 support the view that spermatozoa from the fall copulations are responsible for the activation of the ova, which are released from the ovaries in the spring. Nevertheless, Guthrie and Jeffers ('38) have more recently carried out observations leading them to deny that spermatozoa held over from the matings of fall are capable of fertilizing ova released in the spring. The conclusion reached by these investigators is based, however, upon an indirect method of approach to the problem. They induced ovulation by the injection of hypophyseal extract in bats collected from hibernation in December, January, and February. The failure of ova released during December and January to develop, as compared with the presence of development in ova released during February, led Guthrie and Jeffers to conclude that spermatozoa from the copulations of fall are unable to fertilize

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the ova normally released in the spring, and that new spermatozoa from winter and early spring copulations are necessary to effect fertilization. Yet the earlier studies of Gates ('36) and the more recent studies of Folk ('40), as well as my own previous ones (Wimsatt, '42), employing a more direct procedure, indicate that the assumption of the older investigators is probably correct.

The question can be definitely settled only by isolating inseminated females in the fall to obviate possible winter copulations, and subsequently examining their reproductive tracts in the spring for the presence of spermatozoa and developing ova. This, in essence, is the method adopted in the present investigation, and the results obtained leave no doubt that spermatozoa stored within the female reproductive tract of the bat since the fall do maintain their capacity to fertilize the ova discharged from the ovary in the spring.

MATERIAL AND METHODS

The species used in these experiments were the little brown bat (Myotis l. lucifugus) and the big brown bat (Eptesicus f. fuscus), both belonging to the family Vespertilionidae. The specimens were collected semi-monthly during the winters of 1940–1941 and 1941–1942 from Aitkin and Woodward caves in central Pennsylvania, favored hibernating places for these species. Observations are also included on a single individual of Myotis sodalis, a closely related species.

The bats were collected in the fall soon after the onset of normal hibernation. The females were immediately segregated from the males and placed for the winter in specially constructed hibernating cages. In the spring, at the end of the dormant period, the females were returned to normal laboratory temperatures, under the influence of which they became active and began to take food. At variable periods thereafter the animals were killed for the study of their reproductive tracts. At the time the animals were collected in the fall hibernation was in no case interrupted for more than the 3 to 10 hours required to transport them to the laboratory and to place them in the cages.

The methods of artificial hibernation used, and the success obtained, will be described in some detail, in the hope of saving others who may undertake work on hibernating bats considerable time in working out a technique for successfully maintaining the animals over the winter.

The bats collected during the winter of 1940–1941 were confined in 10″ × 10″ × 18″ wire cages which were placed in water pans, the wire bottoms of the containers touching the surface of the liquid. The humidity was further augmented by damp towelling placed over a portion of each cage. The cages were kept in an electric refrigerator where a temperature of 38° to 42° was constantly maintained. Since they were kept too long in hibernation before being removed to higher temperatures and fed, many of the first year's animals, having used up their reserve of stored fat, died of starvation in the spring. Of the few that did survive, only two were found upon autopsy to contain fertilized ova.

During the winter of 1941–1942, therefore, further attempts were made to improve the conditions under which the specimens hibernated. A small number were treated as in the previous year, but the majority were placed in a wire-enclosed cage, roughly 3′ × 5′ × 7′, which rested on its narrower side. A layer of burlap was tacked outside the screening, and the burlap covered by a layer of tarpaper. On a rack suspended horizontally beneath the roof were stretched two layers of turkish towelling with a cotton filler between. A 5-gallon container of water was so arranged that the liquid dripped upon the towelling from the hoses of a multiple siphon, which were inserted in holes within the solid roof over the stretched cloth. The flow of water was regulated by clamps placed upon the terminal branches of the rubber tubing. The entire apparatus was moved into a refrigerated room where the temperature was kept constantly between 40° and 44°F. The burlap covering soaked up moisture and the roofing paper prevented its rapid evaporation and effectively darkened the interior. The bats placed in this environment had considerably more freedom to move about and change their positions from time to time than those kept in the smaller cages of the previous year. Although many showed weakness and succumbed even under these conditions, the percentage of mortality in the larger cage was well below that in the smaller cages.

A further refinement in handling the experimental animals of the second year, and one which no doubt contributed significantly to the better success of the 1941–1942 experiments, lay in the treatment of the animals after hibernation was ended. In the experiments of the first year (1940–1941) the animals were removed to room temperature and here retained in the small cages in which they had spent the winter. The food offered them at this time consisted entirely of beef liver, which proved to be an insufficient and non-sustaining diet. This combination of lack of exercise and a deficient diet induced a noticeable weakness, from which many of the animals died. In the spring of 1942, therefore, the animals were removed from hibernation to large, roomy cages measuring 3½′ × 3′ × 4½′ completely enclosed by wire. These offered ample room for running and flying about and in the evening the bats were observed to be extremely active. The animals were offered a varied diet consisting mainly of mealworms and cut up honey bees. These diets they accepted, exhibiting a preference for the mealworms. Under these conditions many of the bats finally emerged from hibernation in a fairly vigorous and healthy state, and the normal physiological processes of these animals apparently suffered no significant impairment (except as noted later for M. l. lucifugus).

OBSERVATIONS

Length of life of spermatozoa stored in the female bat

Most previous investigators have expressed the belief that the sperm of autumnal matings are able to survive within the female genital tract over the winter, but until the experiment of Folk ('40) no one had adduced any direct proof of the correctness of this statement.[2] In order to secure further direct evidence of such survival, and to obtain some concept of sperm numbers and activity, those isolated females which began to show signs of weakness were killed at intervals through the winter, and preparations were made, in physiological saline solution, of the scrapings obtained from the opened uterus. These were examined under the microscope for the presence or absence of spermatozoa. Almost invariably such preparations contained great numbers of highly motile sperm.

Table 1 summarizes the data for the twenty-three animals so examined. Since these segregated females had no opportunity to copulate, the table indicates that abundant active spermatozoa were present in the uterus for a period of from 92 to 159 days (over 5 months) following isolation. The presence of only a few or no active spermatozoa in four specimens is probably attributable in three instances to a delay in autopsying the animals. Only in one specimen, isolated 132 days, were spermatozoa few in number for no known reason. In the table the short dagger represents the condition in a closely related species of cave bat, M. sodalis, of which only a few individuals were collected. Only one specimen of E. f. fuscus (designated in the table by an x) was examined for the series presented in table 1, but this uterus contained active sperm, a finding which is in keeping with the previous observations of Folk ('40) and Wimsatt ('42), as well as with results which will be presented below.

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[2] See the experiments of Folk ('40) and Gates ('36), summarized in my earlier publication.

TABLE 1

Direct observations on length of life of spermatozoa in the female genital tract.

SPERMATOZOA IN UTERUS	DAYS IN ISOLATION OF FEMALE BATS													
	92	113	125	126	128	129	130	132	133	135	136	141	156	159
Abundant and active	* ..	* ..	* ..	* ..	* *	*** **	.. ..	.. ..	* ..	.. ..	x ..	* ..	* ..	.. ..
Abundant active and inactive	..	..	..	..	..	...	*	..	..	..	..	..	..	*
Moderate active	..	..	..	..	..	...	..	..	..	*†	..	..	..	..
Many inactive and a few active	..	..	..	*	..	...	..	..	..	..	..	..	*	*
Few and inactive	..	..	..	..	..	...	..	*	..	..	..	..	..	..

* Myotis l. lucifugus † Myotis sodalis x Eptesicus f. fuscus

Fertilizing capacity of spermatozoa stored in the female bat

While the longevity of spermatozoa in the female reproductive tract of the hibernating bat can no longer be doubted, the ability of these spermatozoa to initiate normal development has been questioned by Guthrie and Jeffers ('38).

The present results with M. l. lucifugus, although they establish that the stored sperm are capable of successfully fertilizing the ova, are few in number, due primarily to the difficulties involved in keeping this delicate species alive and vigorous under artificial conditions. In the spring of 1941 no conceptions were recorded for isolated specimens of Myotis, but in 1942 five were found to possess fertilized ova in early stages of development.

In the sturdy big brown bat (Eptesicus), however, the experiments were numerically more successful. In 1941 two instances of conception were recorded among seven bats surviving hibernation. In the experiments of the subsequent year, employing the improved procedure of maintaining the animals, twenty cases of conception occurred among twenty-five individuals which successfully survived the period of hibernation.

Forty Eptesicus females were isolated in 1942, as against nearly 200 Myotis. The greater percentage of success in Eptesicus in contrast with Myotis was attributable not so much to the greater differential mortality rate in the latter as to the fact that large numbers of Myotis had not yet ovulated at the time of autopsy. Specimens of Myotis will ovulate spontaneously within several days if brought from the caves to room temperature any time after the middle of February (Guthrie, '33;

Guthrie and Jeffers, '38). The fact that large numbers of my specimens did not ovulate in April and May, even after 2 or 3 weeks of activity at room temperature, appears to indicate that the artificial conditions created, although sufficient to keep the majority of bats alive and in a seemingly healthy condition, were in a great number of individuals unfavorable in some way for the normal preovulatory swelling and rupture of the graafian follicle. My own series of pregnant Eptesicus was augmented by the unsectioned entire genital tracts of eight specimens very kindly sent to me by Dr. Donald Griffin of the Biological Laboratories at Harvard University. The females from which these tracts had been removed had been isolated for various lengths of time before being killed, and some of the longer records of sperm survival here presented are from these bats.

The procedure for establishing whether or not the spermatozoa stored until spring are capable of fertilizing the ova differs from that adopted for those killed to establish the length of sperm survival. In the bats utilized for the latter purpose the uterus was destroyed in the process of obtaining the spermatozoa, while in the present procedure the entire genitalia were sectioned serially for histological examination.

Table 2 presents the significant data on the five specimens of Myotis and the thirty specimens of Eptesicus which yielded positive evidence that fertilization had taken place. Of the latter genus the table includes the two specimens of the 1941 group, the twenty of the 1942 group, and

TABLE 2

Results of experiments on fertilizing ability of spermatozoa stored in the uterus of M. l. lucifugus and E. f. fuscus.*

DEGREE OF EMBRYONIC DEVELOPMENT	DAYS IN ISOLATION OF FEMALE BATS													
	21	22	25	35	37	38	92	137	138	143	144	154	155	156
Cleaving tubal ova	..	..	5	*1	..	5, 3	..	*1	*1	3, 3	..	...	..	...
Cleaving uterine	4	4	..	..	3	4	..	*1	..	2, 2	4	5, 4	..	3
ova	..	..	..	..	..	...	..	..	..	...	..	3, 4	..	...
Free uterine blastocysts	..	..	..	..	..	...	3	..	*1	...	4	4, 2	4	...
Implanting blastocysts (early)	..	..	..	..	..	...	5	..	..	...	..	4	..	...
Implanting blastocysts	..	..	..	..	..	...	..	..	..	...	..	3, 6	4	4, 3
(late)	..	..	..	..	..	...	..	..	..	...	..	...	..	5

* M. l. lucifugus is represented by asterisks, E. f. fuscus by plain numerals. The denomination of the numeral in each case represents the number of developing ova found in the reproductive tract.

the eight animals received from Dr. Griffin. The results presented in the table for M. l. lucifugus are by far the longest known records of the sort for this species. The only other successful experiment reported for Myotis, in two specimens each of M. sodalis and M. l. lucifugus, is that of Gates ('36), summarized in my earlier publication.

In M. l. lucifugus a single ovum is characteristically released from the ovary, and this species is represented in the table by asterisks. In E. f. fuscus, however, a variable number of ova may be released from the ovary, and the number of ova observed in each of the experimental animals is indicated by the appropriate numeral. All ova in a given specimen had undergone approximately the same degree of development. The table obviates the necessity of describing the results in more detail. Anyone interested in the specific details of treatment of the specimens may obtain this information from the writer by direct inquiry.

From the evidence presented the general conclusion may be drawn that in the North American hibernating bats, E. f. fuscus and M. l. lucifugus, spermatozoa introduced into the uterus of the female at the time of copulation in September and October are able to remain functionally alive within this organ throughout the hibernation period of several months. Furthermore, the stored spermatozoa may, under natural conditions, initiate normal development of the ova released from the ovaries in the spring. The role of spermatozoa from winter and early spring matings will be considered in the discussion.

DISCUSSION

The information obtained from direct observation and vaginal smears in the present study, and the data collected by many other workers using similar methods, definitely show that in hibernating vespertilionid bats copulation occurs in the fall. There is also definite proof that supplementary copulations occur during the winter and early spring. Duval (1895), Rollinat and Trouessart (cf. Hartman, '33), Robin (1881), and Caffier and Kolbow ('34) report spring matings for European species. In America Guthrie ('33) has reported that Myotis copulates during the spring, and I have made similar observations in the Pennsylvania caves.

There are authors, notably Mathews ('37) and Grosser ('03), who deny the possibility of winter and spring copulations. Their conclusion is based upon the presence of a "plug" within the vagina during hibernation. This obstruction is said to be composed either of male glandular secretions (Rhinolophidae), or of desquamated cornified epithelial cells from the wall of the vagina (Vespertilionidae), and is held by these investigators to be the best proof that spermatozoa stored since fall activate vernal ova.

Mathews concludes that in the British horseshoe bats winter copulations are not possible, since the vagina is obstructed by a plug formed from secretions of the male urethral gland. He describes the plug as having the consistency of soft cartilage. He likewise states that there is no evidence that spring matings occur in this species, and he discusses specimens in which ovulation had taken place and development progressed to the blastocyst stage without the plug being lost. Grosser reports a "fibrous atresia" of the upper vagina and cervical canal in Vespergo noctula (Nyctalus noctula). According to Grosser, following copulation in the fall the cornified epithelium of the upper vagina sloughs off completely into the lumen to form an epithelial plug which remains in place throughout hibernation and supposedly prevents further matings. Courrier ('25) noted a similar vaginal plug in the common European pipistrelle. According to both authors the plug is cast out in the spring and ovulation is followed by fertilization of the ova by stored spermatozoa.

Reeder ('39) reports encountering an epithelial vaginal plug only irregularly in M. l. lucifugus. My own observations on this species corroborate her findings. Whenever encountered, the plug was transferred to a slide, broken up, and examined. It consists almost entirely of a white to yellowish cheesy mass of cornified epithelial cells and sometimes considerably distends the vagina, through the thin walls of which it is easily visible. Leucocytes rarely occur within it, but a few spermatozoa are occasionally found. I am unable to explain why the plug is present in so few of the hibernating bats of the species examined by Reeder and myself. Nor, in view of the observed winter copulations in Myotis, is it clear whether or not the os penis is capable of penetrating such a mass. These winter matings may of course only involve those females without the plug. This suggestion is borne out by the fact that I have not observed vaginal plugs in any of the bats observed to copulate during winter. In any event, the vaginal epithelial plug is not a constant feature in M. l. lucifugus or E. f. fuscus, and winter copulation, at least in the former species, may occur under natural conditions.

Since winter matings are known certainly to take place, Caffier's ('34) and Hartman's ('39) assumption that development of ova released from the ovary following prolan injections in February is initiated by spermatozoa of the fall inseminations is not necessarily correct, since the females which were not isolated may possibly have copulated at any time previous to collection.

Now that winter and spring copulations as well as the original fall matings are definitely known to occur in certain hibernating bats, we may well inquire into the significance of such supplementary coitions. Do they serve to fertilize immature females born the previous spring, as certain earlier workers supposed? If there are species capable of supplementary copulations during the winter, in which the young females attain sexual maturity after the fall inseminations, this might be a logical consideration, but Guthrie ('33) holds that young females of M. l. lucifugus reach sexual maturity during their first summer, and could, consequently, be inseminated during the fall. Are supplementary copulations necessary to fertilize the vernal ova? There are two aspects to this question: Do the spermatozoa from the fall retain their fertilizing capacity under normal conditions? We know from the present experiments that they do retain this capacity; but what are the respective roles of fall and later, supplementary matings in reference to fertilization of the ova in the spring? Guthrie ('33) has indicated that recurrent periods of activity, due to temperature changes during the time of hibernation, may cause a diminution in the number of the spermatozoa to the point of infertility as the result of their ingestion by leucocytes, or their extrusion from the tract by muscular contractions. Guthrie and Jeffers ('38) concluded that the sperm of the fall inseminations in M. l. lucifugus are incapable of initiating the development of ova released by artificial means from the ovaries in December and January, and that new spermatozoa of later matings are necessary for fertilization of the eggs. The observations recorded in the present paper show that this is not the case, because we have demonstrated that the sperm surviving from the fall matings are able to fertilize the ova extruded from the ovaries in the spring.

Although winter matings are definitely known to occur, it is entirely possible that many gravid females of late spring have not participated in the supplementary breedings. This possibility is suggested by field observations. I have oftentimes noticed that individual bats may hang motionless in the same spot for a month or more during hibernation, while the groups of animals around such individuals may change their positions between every fortnightly visit. It seems unlikely that such stationary individuals have copulated, at least while in their apparently uninterrupted period of dormancy.

In conclusion, therefore, we may summarize the relative significance of fall and winter matings in this way: Fall inseminations take place as a physiological response to estrus before and as the bats enter the caves for hibernation. Guthrie ('33) states that the earlier fall copulations are not conditioned by the presence in the ovaries of intermediate or large follicles. Yet is should be pointed out that in mid-October, at a time when fall inseminations are still going on, the ovaries of most female M. l. lucifugus examined contain a conspicuous graafian follicle. This follicle, which may be found in either ovary, is destined to remain practically unchanged until ovulation occurs concomitant with resumed activity in the spring. The presence of the graafian follicle and the histology of the reproductive tract in general indicate that the fall estrus continues throughout the period of hibernation and ends only with the rupture of the follicle in the spring. On this assumption, the incidence of winter copulations among the hibernating bats during irregular periods of activity, brought on by temporary seasonal changes or by other disturbing factors [3] can be interpreted as being due to the same physiological course which gave rise to the fall matings; that is, they are a response in the awakened bat to the influence of estrus. Guthrie ('33) has shown that females kept active for several days at laboratory temperature tend to lose their stored spermatozoa. Although this may also occur in nature, it is unlikely that the majority of individuals remain active sufficiently long to cause a significant diminution of spermatozoa.

These observations, therefore, lead me to suggest that the inseminations of late winter and early spring, which are probably of irregular occurrence, and which do not necessarily involve all of the females later found pregnant, serve as a means of "multiple assurance" that as many females as possible will receive spermatozoa in the quantity necessary to make fecundation certain. When a female has been so active that her sperm complement is reduced, supplementary matings in winter and spring would serve to restore the lost supply. The important fact is that the fall sperm are able to initiate normal development of the ovum, and in many individuals probably do so, while in the unknown percentage of individuals that mate later, these matings serve to supplement or replenish the supply previously received during the fall.

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[3] Whenever a cave was first entered on a given visit, the majority of bats were observed to be dormant. After a brief time, however, the disturbance created by our presence awakened numbers of the hibernating animals and considerable activity resulted. The large clusters, quiescent at our first approach, would gradually become bustling, squeaking masses of moving animals; some would break away to fly up and down the passages, while others busied themselves scratching and cleaning their fur. However, occasional pairs were observed copulating, a behavior the significance of which is discussed in this paper.

SUMMARY AND CONCLUSIONS

1. Females of two species of bats, Myotis l. lucifugus and Eptesicus f. fuscus, were collected in the fall and early winter, isolated from males, and placed in hibernation under laboratory conditions. Some were later killed while still dormant, whereas others were exposed to room temperature and fed for varying lengths of time before being sacrificed.

2. The genital tracts of those autopsied during hibernation were examined, and the presence of highly motile sperm was noted as many as 159 days following isolation.

3. The genital tracts of those animals which were kept at room temperature for a time before being killed were examined histologically. The presence of normal embryos was observed in all thirty specimens surviving hibernation of a total of fifty-five females of E. f. fuscus, and in five of seven female M. l. lucifugus that had ovulated following prolonged artificial hibernation. The two females which had been separated longest from males and which contained normal embryos were isolated 138 (Myotis) and 156 (Eptesicus) days respectively. It is evident, therefore, that the stored spermatozoa retain their ability to fertilize the ova released spontaneously from the ovaries in the spring.

4. In the hibernating vespertilionid bats of the northeastern part of the United States copulation normally occurs in the fall, and examination of the female genital tract during October invariably reveals the presence of spermatozoa. Supplementary matings, however, may take place at any time during the winter and early spring, when the bats become active due to temporary seasonal changes or local disturbing factors. Evidence cited, however, admits the probability that not all hibernating females later to bear young necessarily experience supplemental copulation.

5. The condition of the ovaries and the occurrence of supplemental matings throughout the period of hibernation indicate that estrus is maintained from the fall throughout the dormant period until ovulation occurs in the spring. Fall sperm are capable of fertilizing ova shed in the spring, yet the supplementary copulations appear to represent a means of ''multiple assurance'' that enough sperm will be on hand to effect conception in the greatest possible number of females when ovulation occurs in the spring. If Guthrie's contention be true (and I have no evidence to the contrary) that young females of M. l. lucifugus reach sexual maturity during their first summer, then these would in all likelihood be inseminated in the fall, along with the older females. In that event, the sole purpose of supplementary copulations would not be the insemination of bats born the previous summer, as many earlier investigators have claimed.

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IN VITRO TRANSFORMATION OF SPINDLE CELLS OF NEURAL ORIGIN INTO MACROPHAGES[1]

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THIRTEEN FIGURES

In the course of extensive studies on nerve growth in vitro, I have repeatedly observed macrophages in numbers much too high to be assumed to have preexisted in the explanted pieces. The possibility that many of these cells might have arisen by transformation from fixed tissue cells under the conditions of explantation thus suggested itself. Closer inspection under favorable conditions has revealed that spindle cells of both spinal ganglia and peripheral nerve fragments of the embryonic chick may change into cells of the morphology and behavior of macrophages. While some of these convertible spindle cells are unquestionably of the type commonly referred to as fibroblasts, the sheath cells (Schwann cells) and their spinal ganglion homologues, the satellite cells, appear to behave in the same fashion. Though most of the observations were made incidental to work on other problems, sufficient evidence has been accumulated to present a consistent account.

The phenomenon is of interest in several respects. Firstly, macrophages play an important role in the regeneration of peripheral nerves. It is their function to remove the debris of the degenerated axis cylinder and myelin from the distal stump (Cajal, '28; Nageotte, '22). No less significant is their proteolytic activity, which insures the presence of liquid spaces in the scar tissue and distal stump, in the absence of which there would be no room for the passage of the outgrowing sheath cells and young axons (Weiss and Taylor, '43). It is quite conceivable that excess, as well as deficiency, of macrophage activity might explain many failures of nerve regeneration. It would seem desirable, therefore, to gain control over the volume of macrophage accumulation which, in turn, presupposes knowledge of their origin and mode of concentration.

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If the existence of intraneural, in addition to hematogenous, sources could be demonstrated, this would obviously be a point of significance.

Secondly, the appearance of macrophage-like cells from cultures of the embryonic brain has been repeatedly reported and has gained some prominence in connection with the discussion of the origin of microglia (Kapel, '27; Olivo, '27; Verne, '30; Wells and Carmichael, '30; Costero, '31; Mihalik, '32). The appearance of these cells from spinal ganglia and peripheral nerve is, therefore, worth recording. None of those authors seem to have observed the phenomenon of transformation. For if they had been aware of it, this would undoubtedly have influenced the interpretation of their results. On the other hand, transformation in vitro of connective tissue cells into macrophages has occasionally been observed, either by accident (Fischer, '27; Parker, '32) or after irritation (Möllendorff, '31). For all these reasons, the phenomenon would deserve a more systematic study than we have been able to accord it, and the present brief account may be considered more as a stimulus to such future research than as a final word on the subject.

MATERIAL AND METHOD

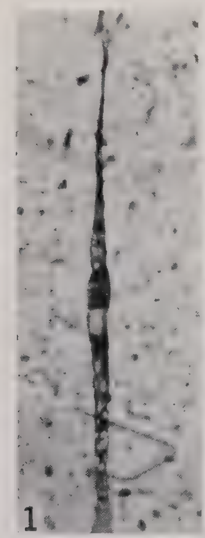
All our observations pertain to spinal ganglia and peripheral nerve fragments of chick embryos of from 10 to 13 days of incubation. For reasons not connected with the present problem, most of these tissues were cultured in liquid medium on the surface of the cover glass. This culture method enhances the transformation, while plasma clot culture is less suitable except in areas in which fibrinolytic liquefaction has taken place. The medium consisted of a mixture of defibrinated blood serum and Tyrode solution in a ratio of 1:5. In some cases a trace of embryonic extract was added. As support for the cultures, cover slips of glass or mica were used. The liquid medium was contained within a paraffin ring of about 1 cm. in diameter, a standard practice in this laboratory, similar to the procedure of Twitty and Bodenstein ('39). In order to secure the adhesion of the explant to its support, in the absence of which growth would not occur, the tissue fragments are transferred from Ringer's solution to the dry cover glass and all excess fluid is blotted off until the tissue fragment sticks. Only then the liquid medium is added cautiously so as not to wash the explant off. The cultures are then covered with depression slides in the usual manner and paraffin sealed. In a number of cases the spinal ganglia were placed in contact with small rods of glass or synthetic resin glued to the surface of the cover glass.

Observations were made on the preserved cultures in total mounts. The cultures, after fixation in Bouin's solution or formalin vapor, were treated according to the protargol method of Bodian. This method gives frequently good cytological definition with differential impregnation of the nucleus, nucleolus and the fibrous elements of the cytoplasm. In order to test the phagocytic activity of the transformed macrophages, carmine was added to some living cultures, which after the ingestion of the granules was observed, were fixed in formalin vapor, stained with hematoxylin, and imbedded in glycerin.

RESULTS

The growth of nerve processes in the cultures may be disregarded in the present paper. Aside from nerve fibers, numerous cells leave the explants and extend along the interface between the solid substratum and the liquid medium. Cells not initially in contact with the cover slip may reach the latter by moving down over the exposed surface of the explant. The migrating cell population consists mostly of two distinct classes, spindle cells and macrophages. The spindle cells are of dual origin. Part of them are of the fibroblast type, coming from the membranous capsule of the spinal ganglion, as well as from the endoneurial connective tissue of the ganglion and the attached nerve stump. The rest are sheath cells and satellite cells. When reared in plasma clots, fibroblasts and sheath cells can usually be distinguished from each other with little ambiguity (Esaki, '29; Murray and Stout, '40, '42). In our experience, too, the fibroblast usually appears as a stouter cell with the nuclear contour rarely touching the cell surface, while the sheath cell is slenderer, of more cylindrical build, with the nuclear diameter frequently reaching clear across the cell, often even bulging the cell contour. Sheath cells, moreover, tend to group in tandem, building up long, filamentous strands similar to those observed in the regeneration of peripheral nerve.

These distinctions, however, lose their sharpness when the cultures are reared in liquid medium. Then, many cells of undoubtedly fibroblastic character begin to resemble sheath cells. They become extremely elongate and their processes impregnate intensely with silver so that an inexperienced observer might even mistake some of them for nerve processes. These cells are presumably only partially in contact with the substratum. When a cell moves down over the surface of the explant and its free pseudopodial end reaches the cover glass, this end may proceed centrifugally, while the proximal end may remain anchored in the explant above the level of the cover glass. The cell body thus spans the liquid space between the two points of attachment, and as the distal end advances the cell is stretched out to great length and becomes correspondingly thinner. Ultimately, the proximal end may tear loose and the cell resume more normal proportions. This mechanism of locomotion explains the presence of nests of free or anastomosing spindle cells found after a few days of cultivation at some distance from the original explant in liquid medium, or in liquefied parts of plasma clot cultures. Even though fibroblasts in liquid medium may thus simulate the appearance of sheath cells, it is reasonable to assume that not all cells of this appearance are overstretched fibroblasts but that many of them are real Schwann cells. Figure 1 illustrates such a cell from one of our cultures.



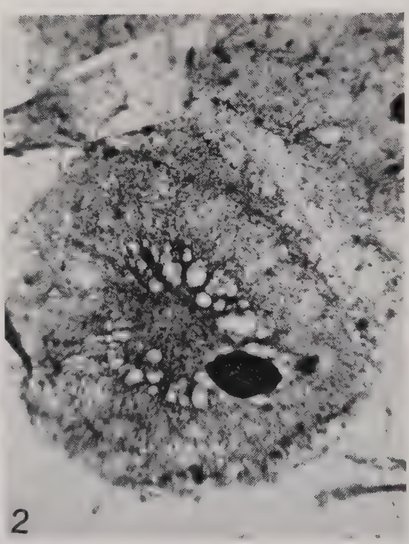


Fig. 1 Spindle cell of sheath cell type. Spinal ganglion, 14-day embryo, 3 days in vitro. × 630.

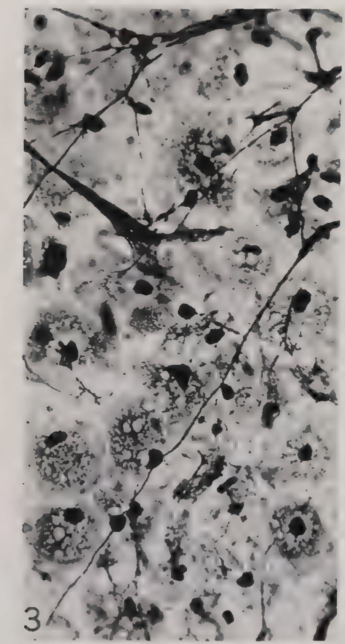
Fig. 2 Transformed macrophage. Spinal ganglion, 12-day embryo, 3 days in vitro. × 630. Note eccentric nucleus, its surface covered with fat globules.

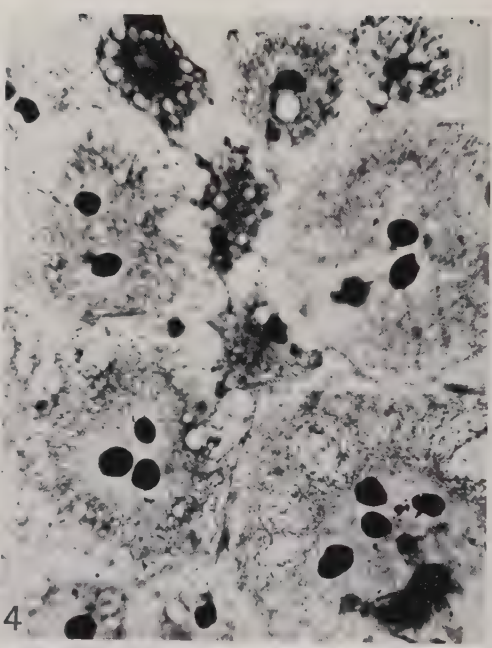
The macrophage-like cells include a great variety of cell shapes and sizes. Some of them, with compact nucleus and large numbers of fat globules, are presumably degeneration stages of other cell types, and some of these resemble the contracted fibroblasts which have been observed in tissue cultures after heat irritation (Kokott, '31). Others, however, are definitely vigorous and healthy, and the following description refers only to this latter group which is composed of large, flattened, disk-shaped cells with a tendency to join into epitheloid membranes. Figure 2 shows one of these cells at the same magnification as the sheath cell of figure 1. Evidence that figures 1 and 2 represent merely different states of the same cell will be presented below.

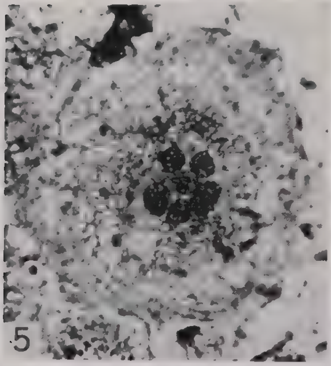
Before discussing their origin, a brief description of the characteristics of these macrophages may be given. They vary considerably as to cell size, nuclear size, number of nuclei, and fat content. The expanded cell shows three distinct zones: (1) a granular central area, surrounded by (2) a reticular zone containing big vacuoles and (3) a more homogeneous peripheral fringe, presumably the homologue of the undulating membrane of the common macrophage. In cells with low fat content, the nucleus assumes a central position. With increasing accumulation of fat globules, the latter tend to move into the center crowding the nucleus into an eccentric position. The fat droplets, recognizable in the living preparation by their high refringence, always appear first on the surface of the nucleus. This furnishes an instructive demonstration of the participation of the nucleus in the production of intracellular fat. Large fat globules often indent the nucleus, sometimes to the point of fission.

The border of these cells is fairly smooth and adheres intimately to the cover slip. At points of local detachment, it retracts, indicating considerable elastic tension in the radial direction. Correlated with this is a striking arrangement of fibrillar elements of the cytoplasm. Fibrillar arrays of oriented ultramicrons tend to show up after silver impregnation as fine striations. In migrating fibroblasts or sheath cells these striations are oriented in the direction in which the pseudopodia are advancing, presumably as a direct result of flow and elastic tensions created by the advance of the cell process. In the round cells here under discussion the striation assumes a predominantly radial pattern, indicating radial tensions in the cell body arising from the expansive tendency of the pseudopodial border. Interfacial tensions along the three phase boundaries between cover slip, exoplasm and liquid medium may be assumed to provide the force for the enlargement. Wherever the cell border becomes detached, however, elastic tensions within the cytoplasm change the ultramicronic orientation from a radial into a tangential course, or more precisely, into lines connecting the nearest points of attachment of the edge.

Besides these large, round forms (fig. 3) there appear smaller ones with more irregular polygonal contours and no centric symmetry, which presumably represent specimens of the former type which have failed to become attached along most of their margin or which have lost their attachment and contracted under the prevailing elastic tensions. Figure







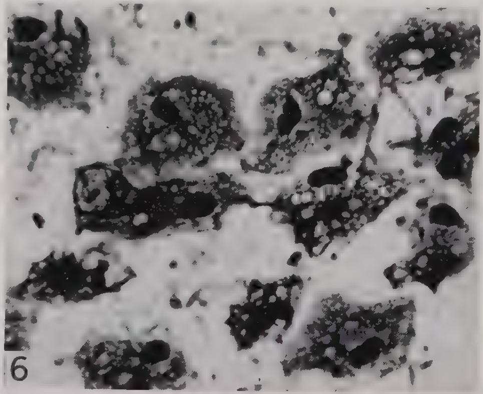


Fig. 3 Group of transformed macrophages amidst regenerated axons; one spindle cell is in the act of transformation. Spinal ganglion, 12-day embryo, 2 days in vitro. × 230.

Fig. 4 Group of transformed macrophages of different sizes, some multinucleate. Spinal ganglion, 12-day embryo, 3 days in vitro. × 450.

Fig. 5 Large multinucleate macrophage. Spinal ganglion, 12-day embryo, 2 days in vitro. × 450.

Fig. 6 Smaller macrophages. Spinal ganglion, 12-day embryo, 3 days in vitro. × 450.

6 illustrates this type; figure 4 shows them interspersed with expanded disk-shaped cells. As both forms occur side by side in the same cultures, it is probable that we are dealing with trivial local differences of attachment rather than with cells in different states of nutrition or age.

Cells of this description can be found in cultures as early as on the second day after explantation. Though moving singly, they tend to stick together following accidental contact. In this manner they aggregate into small epitheloid patches, one of which is illustrated in figure 3. No evidence has been seen that any directive force or mutual attraction, other than is afforded by mere chance impact, is instrumental in producing the observed aggregation. When two cells meet, common surface tensions tend to draw them together even further, which leads to the straightening of the line of mutual contact, comparable to the flattening of the area of contact between two bubbles in a foam, two blastomeres, or two epidermal cells. The border line between two joined cells can be clearly recognized in figure 2 as a fine row of silver granules. In a case like this the two cells are in intimate contact but have not become confluent.

Other instances, however, furnish just as convincing a demonstration of complete protoplasmic fusion among two or more such disk-shaped cells. In such cases the boundary breaks down, and the content of the cells redistributes itself in such a fashion that their former central zones, including the nuclei, merge into a single mass in what now is the center of the whole complex. In cells with low fat content, the several nuclei will form a nest in the center of the cell, as is illustrated by the six nuclei in the giant cell of figure 5, and the two, three and five nuclei, respectively, of the multinuclear cells of figure 4. With the accumulation of more fat, however, the nuclei are gradually forced into a more peripheral position. The maximum number of nuclei observed in a single cell was fifteen, and the diameter of the largest cells was 0.1 mm. These giant cells resemble both in origin and appearance those previously obtained in tissue cultures of the spleen (Lambert, '12), lymph nodes (Lewis and Webster, '21; Maximow, '23) and Jensen's sarcoma (Fell and Andrews, '27); compare also figure 127 of Levi ('34). Most of these earlier observations indicate a dual origin of giant multinucleate cells, some resulting from the merger of preexisting cells, others from amitotic nuclear multiplication without concomitant cell division (Barta, '25; Lewis, '27). I have found no reliable evidence in my preparations of amitotic or mitotic divisions in these cells although the pinching off of nuclear fragments under the pressure of surrounding fat globules has been observed rather frequently. It must be considered, however, that our observations, in contrast to most of the earlier ones, were made in fresh cultures within the first days after explantation, and that if they had been continued for longer periods through successive passages, amitotic divisions might have appeared.

A third cause of the multinucleate condition, peculiar to the present material, lies in the fact that often more than one cell contributes to the formation of these disk-shaped macrophages from the start. This brings us to the cardinal point, namely, the origin of this cell category by transformation from cells of the spindle type. The stimulus for the conversion seems to lie in the contact with the smooth surface of the glass or mica, combined with lack of factors calling forth linear extension.

Figure 8 shows the process under way. This case happens to be that of a peripheral nerve fragment after cultivation for 7 days in a Maximow type of culture. The dark mass on top is the nerve trunk. Extending downward from this, one recognizes ten cells, each with a narrow stalk and a circular terminal disk. These mushroom-shaped bodies are spindle cells whose advancing tips have grown down along the circumference of the nerve and then passed on to the cover glass. On impact with the glass, these tips became flattened out. From this stage on they continue to enlarge under the action of interfacial tensions along the glass surface, and the suction thereby exerted upon the stalk-like portion eventually leads to the detachment of the latter from its hold on the nerve and its incorporation into the flat disk. Once the amoeboid distal tip has made intimate contact with the glass surface, the rest of the cell is literally syphoned out from its mother tissue into the spreading part. Figure 7 gives a diagrammatic view of five successive stages of this process.

An equally prompt transformation occurs, as a rule, at the underside of a spinal ganglion, where it is in contact with the glass support. Lifting a ganglion after several days of cultivation, one frequently finds underneath nests of disk-shaped macrophages which stick to the glass. In most cases, however, the transformation occurs less immediately; that is to say, the spindle cells, either singly or, more often, in reticular groups, migrate from the culture and move about in the medium for some time before undergoing transformation.

Figures 9 to 13 show samples of the transformation process as observed on the second or third day of cultivation. Figure 9 shows an early stage. An anastomosing group of spindle cells has become attached locally to the cover slip, and in this region the cell protoplasm begins to flow out in numerous directions in broad pseudopodia. The plasmodial mass contains four nuclei and is still in connection with other spindle cells of the original network, some of which may later join the syncytium, while others will break loose and continue as spindle cells. At the far end of one of these cells, one notes a second attempt at transformation which may or may not remain abortive. The fact that these local plasma masses contain several nuclei even as they form, indicates that the spindle cells from which they have arisen have been in protoplasmic continuity. Such continuity is generally denied for the fibroblasts of ordinary cultures. It is rather generally conceded, however, to the strands of sheath cells appearing in nerve regeneration, so-called Buengner's cords (Schwannian cords). Although Holmes and Young ('42) have presented evidence that many such cords commonly rated as syncytial are, in reality, composed of individual cells, and, on the basis of this evidence, question the occurrence of syncytial Schwann cords, I have in my study of nerve regeneration in the rat seen many convincing examples of multinucleate Schwannian cords. The present observations in tissue culture amount almost to an experimental demonstration of this fact.

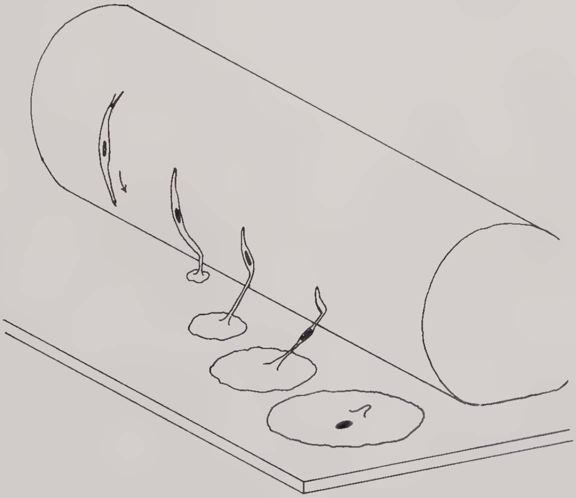


Fig. 7 Five stages of the transformation of a spindle cell from peripheral nerve into a macrophage.

Figure 10 shows the transformation of an isolated pair of spindle cells in which one end has already assumed the typical configuration of the macrophage with central zone, fat globules, and enlarged nucleus, while the other half is still in the elongate state. Figure 11 shows the almost completed transformation of just one cell in a corner of an anastomosing spindle cell network; figure 12, a beginning and a nearly completed terminal transformation; and figure 13, another example of lateral transformation.

The place where the transformation sets in bears no relation to the position of the cell nucleus. The nucleus may be near or far, and depending on its distance, be early or late in gliding from the elongate into the transformed portion (fig. 7). If the cell band contains more than one nucleus, they glide into the disk in succession. Cases of this sort are particularly instructive in demonstrating the primary protoplasmic continuity within such multinucleate bands, in contradistinction to other cases in which there is secondary confluence after the transformation has taken place.

What proportion of the macrophages surrounding an explanted spinal ganglion is contributed by preexisting macrophages of the explant which have merely migrated out, and what proportion by transformed spindle cells, cannot be decided from our experiments. However, in instances where the number of macrophages present is excessive, transformation must have been the major source. In one sample culture of a small spinal ganglion, after 2 days of explantation, 1300 macrophages of all sizes were counted, a figure obviously in excess of what might reasonably be assumed to have been present in the original explant.

As was mentioned before, the extremely flattened, disk-shaped forms of macrophages have appeared only in liquid media or liquefied portions of plasma clot cultures. Moreover, macrophage density is noticeably greater in liquid media as compared with more solid plasma clots and, in fact, macrophages in the latter lie preferentially in the surface of the clot or at the boundary between clot and cover slip, where the physical conditions more nearly resemble those prevailing in liquid medium cultures. The dependence of macrophage density upon the

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Fig. 8 Transformation into macrophages of spindle cells migrating from the side of a peripheral nerve, as in figure 7. 15-day embryo, 7 days in vitro. × 120.

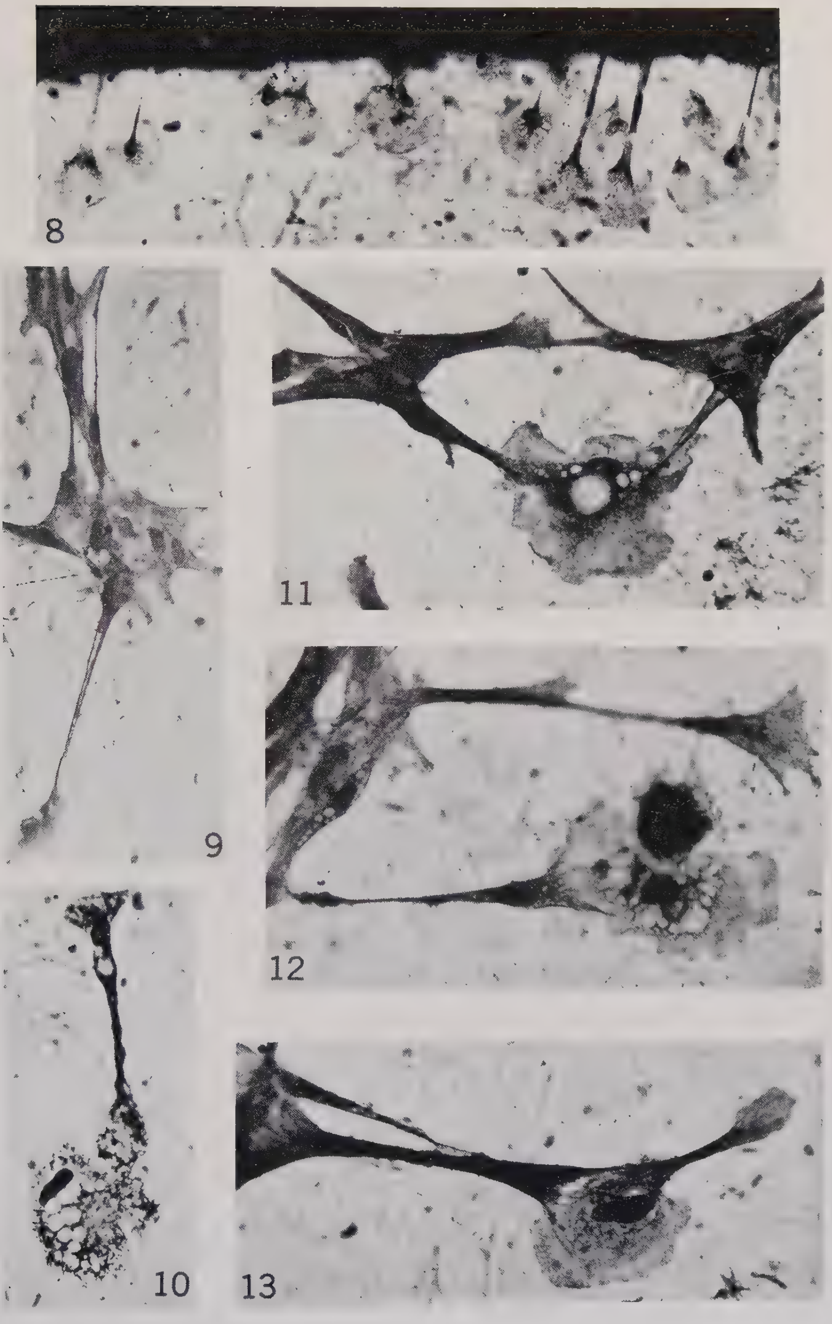
Fig. 9 Early transformation stage of multinucleate cell band. Spinal ganglion, 11-day embryo, 3 days in vitro. × 230.

Fig. 10 Transformation stage. Same culture as in figure 9.

Fig. 11 Transformation stage. Same culture as in figures 9 and 10. × 340.

Fig. 12 Early and advanced transformation stage. Same culture as in figures 9–11. × 420.

Fig. 13 Early transformation stage. Spinal ganglion, 12-day embryo, 3 days in vitro. × 450.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Figures 8 to 13

consistency of the medium can be readily demonstrated by the following experiment: a segment of peripheral nerve is placed into a culture consisting of two different media, one end being embedded in a blood plasma clot while the other end rests on the cover slip bathed in liquid medium; a narrow strip of paraffin keeps the two media from mixing. In two cultures prepared in this manner, macrophage density after 2 days was distinctly greater on the liquid side, and in one culture all macrophages were confined to that side. This result indicates that the liquidity of the medium not only favors the migration of existing macrophages but actively promotes the transformation of spindle cells into macrophage types. If the stimulus for this transformation is what it seems to be, namely, contact with extended smooth surfaces, it is evident that the opportunity for this occurrence would be greatest in a liquid medium where all cells are forced to move along a surface answering this description. The accumulation of macrophages in the surface of plasma clots may be ascribed to their greater buoyancy owing to high fat content.

When a suspension of carmine was added to cultures of the transformed cells, phagocytosis of granules occurred within a few hours. An interesting observation was made on a cell in a stage of transformation corresponding to that of figures 10 and 12. In this cell numerous carmine particles were seen sticking to the outside of the spindly, nontransformed part, while the flat disk-shaped part already contained numerous ingested granules. This case would seem to indicate that the cytoplasm of the cell acquires its phagocytic capacity concurrently with its change into the disk-shaped form, and that phagocytosis is a local property that can be exhibited by transformed sectors of the cell irrespective of the fact that other parts of the same cell may not yet possess it. In view of the fact mentioned above, that ultramicronic orientation at the cell margin is perpendicular to the border in the transformed, i.e. macrophagic parts, while it is parallel to the border in the elongate, spindly portions, one is tempted to assume a relation between molecular orientation and the mechanism of ingestion. Radial orientation at the border would facilitate ingestion in that the entrance path would lie along the lines of structural cleavage, whereas orientations parallel to the border would hem the cell surface with a fibrillar barrier to penetration. This carries no intimation that molecular orientation may be the sole, or even a major, instrument of phagocytosis, but its participation in the act is strongly suggested by our observations.

DISCUSSION

Sporadic cases of transformation of spindle cells of the fibroblast type into macrophages in vitro have been reported by Fischer ('27), Möllendorff ('31) and Parker ('32), all referring to blood plasma cultures after repeated transfers or prolonged cultivation. None of these transformations have occurred as regularly or as promptly as those described in this paper. The difference is presumably to be ascribed to our use of a liquid medium, and it is likely that a more systematic repetition of our experiments would yield a method by which transformation can be obtained at will. The only instances in which transformation of fibroblasts into round cells was provoked by a controlled stimulus, namely, Trypan blue or heat irritation (Möllendorff, '31; Kokott, '31), have yielded cell forms of apparently reduced vitality differing from the forms observed in our cultures. Heat treatment leads to contraction and shrivelling of the fibroblast cell body, in contrast to the active expansion by which the macrophages arose in our cases.

The close dependence of the morphology of these transmutable cells upon the composition and physical constitution of the medium cautions against too much reliance on morphological distinctions as a measure of intrinsic differences. It is not improbable that all the observed transformations, when occurring in identical media and under otherwise identical conditions, would yield strains of macrophages that would look alike and behave alike. This would not necessarily mean, however, that the transformed cells would have lost the biochemical identity peculiar of the particular cell type from which they have arisen. The persistence beyond morphological transmutation of intrinsic physiological properties in the descendents of different cell strains in tissue culture has been demonstrated by Parker ('32), and pending proof to the contrary, the same view may be extended to include macrophage transformation. In other words, all observed transformation would merely constitute a physiological adaptation, or change of state, of a particular spindle cell, whether fibroblast or sheath cell, without involving a change in the basic protoplasmic constitution of that cell such as ordinarily accompanies differentiation. Metamorphosis into macrophages would represent a case of "modulation" rather than of differentiation in the terminology suggested by Bloom ('37) and myself (Weiss, '39). A crucial test would, of course, imply reconversion from the macrophage into the spindle cell state, under which conditions fibroblast and sheath cell should resume each its characteristic properties. Such a test has not yet been forthcoming, however, since none of our cultures were allowed the necessary time and conditions for the manifestation of such reversal.

These comments are of interest in connection with some efforts to elucidate the origin and behavior of microglia by tissue culture methods. Maximow ('25) and Kapel ('27) observed the appearance of granulated, round cells in brain cultures. Later Wells and Carmichael ('30) observed in cultures of embryonic chick brain migratory cells closely resembling microglia. Verne ('30) made similar observations in brain cultures of chick and rat embryos, and Costero ('31) confirmed the fact for embryonic human brain. Already Wells and Carmichael had suggested that since the same type of cell could be obtained from cultures of periosteum and limb buds, all had better be considered as part of the reticulo-endothelial system, and Mihalik ('32) after repeating the experiments came to the conclusion that "the wandering cells in the cultures from the central nervous system of the chick (embryos and young animals) are identical with Costero's microglia cells and with the wandering cells of the brain tissue cultures of Veratti, of Kapel, of Wells and Carmichael, and of Verne." He identifies all of them as straight macrophages, and since his pictures accord very well with the ones observed in our cultures, we may add to his list the spinal ganglion and the peripheral nerve. However, while we were fortunate enough to see the act of transformation directly in our preparations, none of the authors working with brain cultures have ever established the exact origin of these wandering cells, but rather took their preexistence in the explanted fragment for granted. In the light of our present observations, the possibility will have to be considered that some of those cells may have been transformed in the process of cultivation.

Turning now to our introductory question regarding the origin of the macrophages in nerve regeneration, our observations are wholly noncommittal. Firstly, while many of the transforming spindle cells seen in our cultures must have been Schwann cells, a crucial demonstration would have to start from pure Schwann cell cultures containing no admixtures of fibroblasts, which frequently simulate sheath cells very closely. While Murray and Stout ('42) may have seen the first stages of such transformation in their pure cultures of human sheath cells, the process did not go as far as in our cultures, and the question thus remains open. Secondly, our observations pertain to embryonic material, and we have no evidence that adult nerve would behave similarly. Thirdly, the observed phenomenon may be peculiar of tissue culture and not occur in the body.

In spite of extensive histological studies on nerve regeneration in the body, I have been unable thus far to ascertain whether or not transformation of mobilized sheath cells into macrophages in injured nerves may occur. Nor have I been able to find references to this problem in the literature except for an observation of Nageotte ('15) which may be pertinent. He observed that silk threads used to connect severed nerve stumps become surrounded by dense accumulations of macrophages and that during subsequent nerve regeneration the sheath cells of this area remain poorly developed within a radius of from 250 to 300 micra; axons growing through this zone remain thin and poorly myelinated, which in turn can be ascribed to the deficiency of the sheath cell development. If the contact with the silk surfaces should have stimulated sheath cell transformation into macrophages, much as did the glass support in our experimental cultures, the local deficit of sheath cells observed by Nageotte would find a ready explanation. Nageotte himself, however, thinks in terms of an inhibitory action exerted by diffusible substances emanating from the macrophages, and this alternative explanation has not been ruled out. It is to be hoped that further experiments may bring clarification of this important problem which has both theoretical and practical significance.

SUMMARY

Cultures of spinal ganglia and peripheral nerve fragments of 12- to 15-day chick embryos, cultured in vitro for 2–3 days on the surface of a cover glass in a liquid medium consisting of 1 part of blood serum and 5 parts of Tyrode solution, produce large numbers of macrophages, many of which can be demonstrated to originate by transformation from spindle cells of the fibroblast or sheath cell type. The transformation occurs in response to contact between the spindle cell and a smooth surface. The resulting macrophages assume disk-shape with extremely flattened body and a diameter up to 0.1 mm. Phagocytic ingestion of carmine granules by the transformed parts of such cells has been observed. Many of the transformed cells are giant cells with several nuclei. Whether Schwann cells of the adult nerve are likewise capable of transforming into macrophages, hence, of supplying part of the macrophages in nerve regeneration, remains to be demonstrated.

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BOOK REVIEW

FRONTIERS IN CYTOCHEMISTRY. THE PHYSICAL AND CHEMICAL ORGANIZATION OF THE CYTOPLASM. Edited by NORMAND L. HOERR. The Jacques Cattell Press, Lancaster, Pa., 1943.

This volume consists chiefly of a series of thirteen papers contributed to a Symposium held in the University of Chicago on November 13, 1942, in honour of Prof. R. R Bensley, the occasion being his seventy-fifth birthday. During the 10 years that had elapsed since his ''retirement'' in 1932, Dr. Bensley had greatly extended his work in cytochemistry, both through his own researches and those of his associates, with results which had widely influenced investigators in this field. The papers form an impressive tribute to the significance, thoroughness and originality of his work. They have been edited by his former associate, Dr. Normand L. Hoerr (now of Western Reserve University), who in a brief ''Foreword'' describes the general course of Dr. Bensley's researches, extending over a period of more than 50 years, and rich in independent and fruitful contributions. The ''Foreword'' is followed by an ''Appreciation'' by Dr. E. V. Cowdry, the accuracy of which will be acknowledged by all who have had the privilege of knowing Dr. Bensley or of working with him. A general review by Dr. Bensley, ''The Chemistry of Cytoplasm'' (first published in Science of October 30, 1942), forms a fitting conclusion to the volume. There is also a photograph showing Dr. Bensley in his laboratory.

All the papers are biochemical or biophysical and their scope is best indicated by their titles and the names of their authors. These are as follows: ''The Chemical Structure of Cytoplasm as Investigated in Professor Bensley's Laboratory during the Past Ten Years,'' by Arnold Lazarow; ''Some Considerations on the Application of Biological Oxidation-Reduction Systems to the Study of Cellular Respiration,'' by E. S. Guzman Barron; ''Ultracentrifugal Studies on Cytoplasmic Components and Inclusions,'' by H. W. Beams; ''Electrolytic Solutions Compatible with the Maintenance of Protoplasmic Structures,'' by Robert Chambers; ''Distribution of Nucleic Acids in the Cell and the Morphological Constitution of Cytoplasm,'' by Albert Claude; ''Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice,'' by E. V. Cowdry; ''Histochemical Analysis of Changes in Rhesus Motoneurons after Root Section,'' by Isidor Gersh and David Bodian; ''Methods of Isolation of Morphological Constituents of the Liver Cell,'' by Normand L. Hoerr; ''Electrolytes in the Cytoplasm,'' by Oliver H. Lowry; ''Fibrous Nucleoproteins of Chromatin,'' by A. E. Mirsky and A. W. Pollister; ''The Ultrastructure of Protoplasmic Fibrils,'' by Francis O. Schmitt, Cecil E. Hall and Marie A. Jakus; ''Mineral Distribution in the Cytoplasm,'' by Gordon H. Scott, ''Studies on Macromolecular Particles Endowed with Specific Biological Activity,'' by Kurt G. Stern.

It is not possible to describe here in any detail the highly varied and interesting content of these papers and the reviewer will confine himself to a brief consideration of some of the more significant observations and certain general conclusions derived from them. Briefly, the problem of cytochemistry may be defined as (1) how to isolate for analysis the essential chemical and structural components of the living cell, and (2) to indicate how these are combined in the actual organization and activity of the cell. In the work of analysis mechanical, optical and chemical methods are used. Evidently any reliable michrochemical analysis requires that the original composition and distribution of the cellular components should be altered as little as possible; and the method of low temperature dehydration (greatly improved in Dr. Bensley's laboratory and discussed in Hoerr's paper) has been extensively used in these studies as the best means to this end. This method is combined with microincineration in Scott's study of the distribution of mineral constituents in cells. Various compounds, especially proteins, are isolated or differentiated by taking advantage of their differential solubility in salt solutions. The relations of particles and other structural elements (mitochondria, Golgi apparatus) to the normal cell-structure as well as the molecular and micellar size of various components are investigated by the ultracentrifugal technique (Beams, Claude, Stern). Ultraviolet absorption is used in the localization of nucleic acid (Gersh and Bodian). The comparative rates of electrophoresis are also used to differentiate colloidal components (Stern). Mechanical trituration at low temperature forms an important part of the method for the isolation of plasmosin and other nucleoproteins (Lazarow, Hoerr, Stern). Ultrasonic vibrations are used for disintegration in some cases, e.g., Streptococcus pyogenes (p. 311).

In several papers the distribution and physiological significance of inorganic salts are considered in some detail (Chambers, Lowry, Scott; also in Cowdry's study of epidermal carcinogenesis). The relation of fibrous proteins to cellular structure is studied with the electron microscope in the work of Schmitt, Hall and Jakus, and with histochemical methods by Hoerr and by Stern. Special interest attaches to the studies on the distribution and rôle of nucleoproteins (papers by Lazarow, Claude, Hoerr, Mirsky and Pollister, Stern). Mention should also be made of the close associations found analytically between proteins and lipins (especially phosphatides), as indicating the fundamental importance of the latter compounds in cellular structure. On the metabolic side special attention should be drawn to Barron's excellent review of the relations between oxidation-reduction systems and cellular respiration. Since the free energy for cellular activity — including the energy underlying the synthesis and maintenance of cell organization — is derived chiefly from oxidations catalyzed by these systems, their fundamental biological significance is evident. Barron points out that in the living cell their activity is controlled by their relations to cell structure; for example, ground tissues are unable to carry out reactions catalyzed by pyridine nucleotides, while tissue slices of the same organ readily do so (p. 63). The synthetic reactions of the cell, which typically require oxygen, are especially dependent on intact structure.[1]

Mirsky and Pollister differ from Hoerr with regard to the presence of fibrous nucleoprotein (which includes plasmosin) in the cytoplasm of the liver cell. They call attention to the fact that in general the proportion of nucleoprotein extractable from cells increases as the relative nuclear volume increases; they also point out that the Feulgen reaction, indicating desoxyribose nucleic acid, is typically not given by the cytoplasm; their conclusion is that nucleoproteins are essentially confined to the cell nuclei. The general physiological importance of the nucleo-

[1] The symbol for natural logarithm, ln, is misprinted as 1n in the formulae on pages 47 and 56.

proteins is evident: the authors ask, "can we find in nucleohistone the chemical basis for heredity?" Hoerr maintains that nucleoprotein is also present in important quantity in the cytoplasm (p. 227). To the reviewer the evidence seems to indicate that while these compounds are present chiefly in the nuclei — and undoubtedly are chiefly synthesized there — their presence in the cytoplasm cannot be altogether excluded. The known dependence of the general cellular metabolism on the nucleus, as shown by the disorganization following enucleation in all types of cell, is itself an indication of their presence in the cytoplasm. Many cells contain extranuclear structural elements or particles which consist largely of nucleoprotein (Nissl bodies, mitochondria, certain granules); there is also the fact from pathology that viruses (which are known to be nucleoprotein) multiply in the cytoplasm of the infected cells. The relation of nucleic acid to synthetic metabolism is clearly an intimate one; recently the addition of nucleoprotein to tissue cultures has been shown to promote proliferation in many cases (cited by Stern, p. 310). The central position of this material in the cell, a universal feature of cellular organization, seems to indicate a special relation to growth and synthesis, although the precise nature of this relation is not understood at present. While the multiplication of nucleotides appears to occur chiefly in the nuclear area, the case of viruses shows that it may also occur in the cytoplasm. The typical separation of the nuclear material from the cytoplasm by a membrane is a significant fact, but this separation is periodically interrupted during the multiplication of cells by mitosis; the association of mitosis with rapid cell growth and development seems to imply a catalytic relation of the nuclear proteins to the cytoplasmic syntheses. Gersh and Bodian show that the chromatolysis of Nissl bodies following axone section is a reversible process; and on the basis of their observations with ultraviolet absorption, as well as with ribonuclease, they are inclined to localize the resynthesis in the cytoplasm. They also cite evidence from tracer studies of Schoenheimer indicating that the ribonucleic acid in cells is in a continual state of flux (p. 182). A definite level of dynamic equilibrium appears to be characteristic of each type of cell; the reversal of the changes in nucleoprotein metabolism is an illustration of a general condition common to all forms of living matter. Synthetic reactions of many kinds are continual in the cell during life, and cease at death; it is well known that the multiplication of viruses requires the presence of these bodies in the protoplasm of living cells, and what is true for the synthesis of these foreign proteins is obviously true also for the synthesis of the proteins and other metabolizing compounds normally present in the cell. One cannot speak of the viruses any more than of these compounds as independently "living."

In conclusion the reviewer cannot resist quoting Dr. Bensley's final paragraph (p. 333): "It is a pleasure also to reflect that the funds at my disposal have never been large enough to tempt me to abandon investigation for the direction of others, and thus to miss in these years of retirement the joys that come, in fullest measure, only to those who satisfy their desire for knowledge by a direct and personal appeal to nature by research."

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BOOK REVIEW

ESSAYS IN BIOLOGY: IN HONOR OF HERBERT M. EVANS, written by his Friends. The University of California Press, Berkeley and Los Angeles. XXVII + 687 pp. 1943.

This volume is a collection of forty-eight articles presented to Herbert McLean Evans, in honor of his sixtieth birthday, by his colleagues (present or past), pupils, and friends in many places. The book is notable for a long list of distinguished contributors, the remarkable diversity of its contents, and an unusually handsome format. Not even the dire fact of an international war has been enough to keep the work within the physical bounds of an ordinary volume; and its nearly 700 pages constitute an impressive personal tribute.

It is natural that a collection of this sort should exemplify the widest range of individual tastes and interests on the part of the many contributors; and as a matter of fact in modern experimental biology there are few provinces not represented by at least one article. This astonishing topical variety was probably welcomed if not actually encouraged by the editors, as reflecting the scope of Evans' own work and interest. Certainly there are not many investigators of this generation who have been an important influence in so many fields. With even the most liberal restrictions on time and space (charitably overlooking the matter of competency) it is obviously impossible to deal separately with all or any considerable number of individual contributions, however valuable or interesting. Therefore, since a book of this type may not receive a wide distribution to individuals, it may be more useful to indicate as briefly as possible the content of the volume as a whole.

The key-word is diversity, both of subject matter and method of treatment. The collection comprises historical sketches, translations from classical works, philosophical essays, review articles, clinical reports, and technical contributions; besides a large number of papers which present the results of current research. If those articles primarily of an historical or philosophical character are set aside, the remainder fall readily within the limits of a single if very broad domain. Rather more than half of the total are concerned with one or more of the almost innumerable aspects of hormone physiology; and these provide a sort of functional center, or macro-nucleus, for the volume. Here we find information (sometimes new, sometimes redigested) on such topics as the sources and chemical nature of hormones, methods of assay, the part played by hormones in developmental processes and in adult functions, and the analysis of such activities at the anatomical, histological and cytological levels. The intricacies of endocrine interrelationships, and the role of hormones in pathological processes and their value in diagnosis, are also well represented. With such abundance and variety it is unfortunate that there was no attempt at a topical arrangement, an advantage which would have been comparatively easy to achieve and a real convenience to the reader. An effort in this direction, at the risk of seeming arbitrary, will favor brevity and greater coherence in what is to follow.

Closer examination brings to light a number of papers devoted to the more general aspects of endocrine activity — in particular, metabolic effects. This group treats of such subjects as endocrines and intestinal absorption, salt and the physiology of the adrenalectomized animal, endocrine factors in carbohydrate metabolism, the kidney and experimental hypertension, etc. Another group is readily assembled which deals with interrelationships, centering mainly around certain pituitary hormones. The following topics are here included: the chemical nature of some of the pituitary gonadotrophins (with special attention to species differences), the source of equine gonadotrophin (the substance is attributed to special ''organs'' differentiated from the endometrium), a comparative study of the action of various gonadotrophins on the ovary, pituitary cytology as affected by castration or thyroidectomy. The much-investigated subject of sex differentiation likewise receives attention. The problems and processes dealt with vary greatly and are often of a very special nature. The following examples are cited: hormones and the expression of genetic pattern in the plumage of birds, sex differentiation in parabiotic salamanders, the influence of sex hormones on the descent of the testicle, the experimental production of pseudohermaphroditism in fetal monkeys. Hormones in relation to the reproductive cycle and secondary cyclical phenomena comprise yet another general category. Under this head may be listed: functional correlations of ovum, cycle and menstruation; hormones in pregnancy and lactation; the regulation of growth in the mammary glands; antler-gonad periodicity in deer; and the hormonal control of sexual behavior.

At this point there remains a small residue not readily assignable to any of the foregoing groups. Some of these articles are mainly of clinical interest, or are concerned with the role of hormones in pathological processes. Subjects discussed are: the significance of hormone excretion in the clinical study of tumors, the distribution and behavior of experimentally induced neoplasms, and clinical methods of estrogen assay. In addition, two papers should be mentioned which are primarily historical in theme, but will be of particular interest to endocrinologists. One of these is a detailed historical development of present concepts correlating hypophysis and pancreas in hypophysial diabetes; the other a lively and concise biographical sketch of Brown-Séquard, the almost forgotten pioneer and prophet of modern endocrinology. It is regrettable that the great length of this catalogue precludes the citation of authors, and it should be understood that many of the topics listed are not actual titles, and are represented in some cases by more than one contribution.

And now, having disposed in summary fashion of the nuclear material, at least a word should be spoken for a rich array of cytoplasmic inclusions. Reference has been made to a number of papers relating variously to the history and cultural backgrounds of biology and medicine, and their presence signifies more than mere indulgence of the special interests of individual contributors. It is perhaps not widely known that for many years Evans was a zealous collector of material on the history of science — biology, physiology and anatomy in particular. In time he assembled a valuable library. Although apparently he found no time to undertake serious work of his own in this field, there is little doubt that originally he planned to do so, and his continued interest in these things must have influenced many of his pupils and friends.

In conclusion, not the least important feature of the book is a complete bibliography of Evans as of August, 1942. The length of the list is amazing, with no less than 369 titles recorded since 1904. It is a great convenience to have thus provided a single key to so vast a body of work. The bibliography is accompanied by biographical data and a portrait.

R. K. Burns, Jr.

BOOK REVIEW

THE EMBRYOLOGICAL TREATISES OF HIERONYMUS FABRICIUS OF AQUAPENDENTE. The formation of the egg and of the chick [*De Formatione Ovi et Pulli*]: The formed fetus [*De Formato Foetu*]. A facsimile edition, with an introduction, a translation, and a commentary by HOWARD B. ADELMANN. Cornell University Press, 1942. p. xxiii, 883. 27 cm. Portr. and 45 facsimile pls. $12.00.

There has long been need of an English translation of the embryological works of Fabricius. Thirty years ago a classical scholar from Princeton, on obtaining his Harvard M.D., was urged to undertake the task, and was ready to do so if it might lead to the Master's degree. But Princeton had lately decided against granting that degree in absentia —Harvard's terms were the abandonment of medicine for a year — and the project was stifled. But no such scholarly outcome as that now before us could have been expected. For this labor of ''over three years'' — well over, no doubt — Dr. Adelmann merits the degree Doctor of Letters. His translation is complete and precise, in English as smooth as if Fabricius's mother tongue. Most important are the 130 pages of notes, which give exact reference for all quotations and allusions made by Fabricius to his predecessors, especially to Aristotle and Galen. To an equal extent these notes record the citations and criticisms of Fabricius by his successors, especially by Harvey in the ''continuation and amplification'' of the work of his teacher. Thus Dr. Adelmann has accomplished his purpose not only to evaluate the contribution of Fabricius, but ''to furnish a key to the appreciation of 17th century embryology as a whole.'' We shall consider whether this work of Fabricius, which astonished von Baer by its emptiness (die Leerheit dieses Buches) was worth the costly effort.

The second or modern period in the history of anatomy began in Italy and, according to Hyrtl, with ''the famous anatomical triumvirate of Vesalius, Eustachius, and Fallopius.'' Among their immediate successors, he ranks Fabricius the most important (der bedeutendste). Hyrtl does not agree with our Puritan divine, Cotton Mather, who, in 1721, had written that ''*Jacobus Carpensis* was the Gentleman who was indeed the first Restorer of the *Anatomick Art*, which *Vesalius* afterwards carried on;'' but the work of Berengario da Carpi needs only to be read to establish the latter opinion. Mather had literally translated the sound judgment of Fallopius. As professor at Bologna, da Carpi in 1521 had published his Commentaria on Mundinus (1056 pp.), and in 1522, at the urgent request of his students, had issued his brief, clear and profitable introduction to human anatomy (144 pp.). At Padua, still earlier, Benedetti had needed and used an amphitheatre for his anatomical demonstrations, which were reported in eight editions of his Anatomice (1493–1528, listed by Tosoni).

In 1537, Vesalius, not yet twenty-three, after his studies at Louvain and Paris, arrived in Italy, the ''homeland of anatomy and art.'' There, among men of liberal mind, he found the most favorable conditions for attaining his goal, — the enlivenment of anatomy. As professor at Padua, he fought not only with Galen, but attacked all the anatomists, ancient and modern, speaking favorably only of the lost art of Egypt. That is the spirit of the Fabrica, ''the greatest medical book ever written'' (Dr. Osler is quoted); and Roth, biographer of Vesalius, adds: — ''Nothing similar to Vesalian research is found in all antiquity; neither Galen, nor Aristotle, but Vesalius, is the founder of the anatomic method.'' These are extravagant opinions, acceptance of which is fatal to a just appreciation of Fallopius, Fabricius, Galen or Aristotle.

Fabricius was born in the little town of Acquapendente, probably in 1533. ''Genius seems to leap out of nowhere.'' It has no gene. ''As a student of medicine, he aroused the admiration of everyone by his exemplary conduct both in and out of school. At Padua, he attended assiduously the lectures of Fallopius, and 'like a busy bee' stored in his notes the wisdom of his master.'' Thus Dr. Adelmann writes in his full and documented biographical introduction. Fallopius of Modena, it may be noted, was not a pupil of Vesalius, whom he surpassed in the excellence of his anatomical observations (Haller's opinion). By chance it seems, Coiter, another student of Fallopius, collected and published the professor's lectures on similar parts (presumably the first treatise on histology since Galen) which Huxley declared must excite the admiration of every modern reader. Freely quoting Dr. Adelmann, we note that Fabricius assisted Fallopius in dissection, and accompanied him on his visits to the sick.

About 1559, Fabricius obtained the doctorate of medicine and philosophy, whereupon he turned with enthusiasm to the practice of medicine and surgery. Fallopius died in 1562; and 3 years later, by authority of the Venetian Senate, Fabricius became his successor in the chair of surgery and anatomy at Padua. His academic career was a brilliant one. Reappointments and promotions followed regularly until, full of years and honors, he retired from active teaching in 1613. He died in 1619, having been ''the most distinguished teacher of the time in the most distinguished school of Europe'' (Osler).

Anatomy at Padua was a pageant, thronged by students from all Europe, who organized in national groups.[1] The steep and gloomy amphitheatre built for Fabricius in 1594, in which the students stood for hours, remains intact, unless misdirected bombs have shattered it. But the teaching often interfered with Fabricius's research. He proposed to give the course in alternate years, or would lecture many times on the eye, or for 2 months on the bones of the head, according to his special interests at the time; whereupon the students would protest. Surgical practice and publication also interfered. A surgeon who described operating on the lip as ''molestissima, periculosa, magna, atque turpissima'' — a last resort even for cancer — ought in that day to have been popular. His patients included dukes and princes, Galileo and Sarpi, and the poor in large numbers treated without fee.

Most important was his superb portrayal of the valves of the veins.[2] the further consideration of which by his pupil Harvey, repaid that brilliant student from

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[1] Evelyn in his Diary describes the session of 1646. Perhaps the first American student to register there was Henry Saltonstall, A.B. (Harvard) 1642. He enrolled at Padua on December 12, 1648, and received the doctor's degree there in 1649.

[2] De venarum ostiolis, 1603, of Hieronymus Fabricius of Aquapendente (1533?–1619). Facsimile edition with introduction, translation, and notes by K. J. Franklin, D. M. Springfield and Baltimore, 1933. 98 pp.

England for his 5 years at Padua. To many writers, Fabricius here appears bound in "shackles of tradition." "The dead hand of the great Pergamite lay heavy on all thought" (Osler). But blot out any conception of capillaries, unseen for lack of microscopes and unsuspected, and consider how Fabricius regarded these gateways as a contrivance against congestion and varicosity, which they certainly are (the single valve of the jugular veins to serve when the head is dependent), and "the heavy hand of the Pergamite" is merely the slow comprehension of newly made observations — the time element in human cerebration.

The embryological treatises of Fabricius were a part of his ambitious plan to produce a "Fabrica" of the entire animal kingdom, with colored plates, natural size. Dr. Adelmann tells the whole story, with bibliographic details. "The formation of the egg and of the chick" though printed posthumously (1621) should precede "The formed fetus" of 1604 (with variant dates). Fabricius described the yolk of the hen's egg as dropping from a cluster (the "ovarium") like an acorn from its cup, with a "cicatricula" marking its former attachment to the stem. It enters the infundibulum (his new term), and is round to roll down the lower uterus (the oviduct), receiving the chalazae, the white, and finally the shell in its descent. During its travels it may be fertilized by a seminal influence from sperm stored in a vesicle, known from its discoverer as the bursa of Fabricius. Of the bursa he provides a particularly crude figure. Little could he suspect that this remarkable structure would be found related to the thymus, and perhaps the source of a secretion to retard premature sexual development.

The formation of the chick is illustrated in 5 plates of 70 figures, mostly unlabeled except for the day of incubation. It is the earliest attempt to picture serially the progress of development in the chick. Breaking open the shell at the large end of the egg, Fabricius sought for the first sign of development, and in perplexity he sifted the literature. Apparently unaware of the recent studies of Aldrovandi and Coiter, he reverts to Hippocrates and Aristotle, who differ. His argument is long and tedious. All the parts of a perfect egg are considered in turn, with the outcome, — "Nevertheless it is my opinion that the chick is incorporated from the chalazae as the material." "His inference is infirm — he is deceived," as Harvey in 1651 proved beyond question, yet without venturing to make illustrative drawings.

Fabricius, adopting Galen's simile, said that at first a keel (carina) is laid down, namely the chick's spine, necessarily preceded by the cord and brain which that spine encloses. It is an obscure passage, but microscopes were becoming available. With their aid, Malpighi (1672) made this carina — for he still used the term — clear as day. Modern embryology was launched.

The title of Fabricius's final embryological treatise, "The formed fetus," is somewhat misleading, since it deals primarily with the mammalian afterbirth. In text and illustrations it is a masterpiece that can never be displaced. The ancients, familiar with the birth of sheep and cattle, had named the chorion, amnion, and allantois, which are by no means microscopic; yet there was confusion in regard to all of them until the publication of this atlas. It provides the first realistic picture of the long, twisted human cord leading to a discoid fleshy mass which Columbus, Fallopius, and Fabricius were then calling the cake or placenta (perhaps a midwife's term). On a large clear scale Fabricius shows the corresponding parts in the sheep, cow, horse, pig, dog, rat and guinea pig.

Galen had described the umbilical cord as containing two arteries and two veins. He had studied the afterbirth of a ruminant, as Fallopius and Fabricius well knew. It needs only casual observation to show that the cord of the pig or of man has only one vein, and Berengario da Carpi, in 1528, reported as follows:

> Galen says that there are two veins in the cord, but on most careful dissection I have seen only one. For some time I believed that there were two, which became one within the abdomen of the child and went on to the liver, because I believed Galen; but I found only one vein in the cord and two arteries, and that's the very truth. Not content with past observations, I wish to see particular dissections, that I may be square with God, myself, and posterity.

Yet Vesalius, in 1543, obviously without investigation, ascribes four vessels to the human cord. Estienne, in his pre-Vesalien studies delayed in publication until 1545, quoted Galen before reporting that he himself could find only two vessels in the human cord — one vein and one artery. He says that he dissected and probed carefully, yet "we hope you will be free to experiment further: we would not be obstinate." Estienne was probably mistaken, and also Paré (1550), who made a similar statement. They saw the three vessels in the cord, but mistook one of the arteries for the urachus. Fallopius, in 1561, having recognized the usual condition, added that once (aliquando) in the human cord he found a single artery and not a pair. Such cases are rare, but Hyrtl secured eight specimens.

At such a time, to have published a little figure of a human fetus within the zonate chorion of a dog must have been, as Dr. Adelmann remarks, a "humiliating experience" for Vesalius. Especially so, since Vesalius charged Galen with "fashioning a chimera" in which he mixed the description of a cow's uterus which he had seen, with that of a woman which he had "never gazed upon, not even in a dream." Yet Vesalius, for all the world to see, has put the aortic arch of a pig (or a dog?) with great veins to match, in his finest plates of the human vascular system. As to the sac-like allantois which Vesalius pictured in man, and both he and Harvey denied in the sheep — "regrettable blunders" all!

The allantois may be defined in two ways. In the opinion of C. S. Minot the allantois is a ventral diverticulum of the entodermal tract, which may expand distally to form an allantoic sac, and proximally to make the bladder, with an epithelial allantoic duct (the urachus) connecting the two. (The allantoic duct is to be distinguished from the allantoic stalk: the latter includes the duct and its connective tissue investment, with the vessels — cf. vitelline duct and yolk stalk). For Franz Keibel on the contrary, as also for the early anatomists, the allantois is the sausage-like sac at the end of the urachus. The bladder is a ventral subdivision of the cloaca. Dr. Adelmann has, perhaps, not made these distinctions sharply enough. He states (p. 747, note 12) "we may excuse Fabricius for having overlooked the rudimentary human allantois." But the human embryo has no allantois in the original sense and in that of Keibel: it has merely an allantoic duct, and for once Fabricius needs no excuse.

In insisting that the residues or waste products of the embryo serve a useful purpose — the urine in the allantois, for example — Fabricius has received an unexpected confirmation. For Boyden has shown, in a brilliant experimental study, that the expansion of the chick's allantois by the contained fluid is requisite to bring its vessels into contact with the chorion, without which the chick perishes for lack of respiration.[3] "It is Aristotle's opinion," says Fabricius,

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[3] Boyden, E. A. The development of the avian cloaca. J. Exp. Zool., 1924, vol. 40, pp. 437–471.

''that every instrument has been made for the sake of something.'' Nature never does anything in vain. Why then, he asks in conclusion, do the umbilical vessels, which have served the fetus so faithfully and well throughout intra-uterine life, meet death and destruction at the time of birth? ''Why do they meet this most unfortunate and lamentable fate?'' They have outlived their usefulness, he reflects, and like gangrenous hands and feet, they call for amputation.

Taught thus by Nature, some modern educators would relieve the medical student, especially in war-time curriula, of giving a thought to Fabricius and his antique labors. ''Forgetting those things which are behind'' — press on! But by turning the pages of this admirable book which Dr. Adelmann has made available in every laboratory, the student, perhaps more quickly and securely than in any other way, may learn what amnion, chorion, and allantois really are — there should be afterbirths also at hand — and may come to know Fabricius, one of the most successful teachers in the history of medicine.

Frederic T. Lewis,
Harvard Medical School.

NERVE FIBERS WITHIN THE CRANIO-PHARYNGEAL CANAL

GLENN A. DRAGER
Institute of Anatomy, University of Minnesota, Minneapolis

ONE TEXT FIGURE AND TWO PLATES (EIGHT FIGURES)

INTRODUCTION

While conducting a comparative study of the innervation of the anterior lobe of the hypophysis, it was observed that a bundle of nerve fibers and blood vessels were constantly present in the cranio-pharyngeal canal of the cat. Because of the proximity of this nerve bundle to the pituitary gland, it was thought advisable to ascertain the origin and distribution of the nerve fibers.

In the majority of mammals the cranio-pharyngeal canal closes soon after birth. However, this passage persists throughout life in some species. This is the condition in certain rodents (guinea-pigs, mice, rabbits and hares), in carnivores (all of the cat species), in artiodactyls (antelopes and hippopotamuses), in perissodactyls (rhinoceroses-Maggi, 1893), in cetaceans (finback whales-Geiling, '35), and in primates (30% of the apes, 3% of the human adults, 10% of human newborns-Landzert, 1868; Maggi, 1890; Rossi, 1891; Kollmann, '04; and Waldeyer, '04).

I wish to express my appreciation to Dr. A. T. Rasmussen for helpful suggestions and criticisms.

MATERIALS AND METHODS

This study was based upon the microscopic examination of the cranio-pharyngeal canal of eight adult cats. A block of the sphenoid bone containing the cranio-pharyngeal canal and adjacent structures — such as the hypophysis and adjacent hypothalamus, blood vessels, nerves and pharyngeal tissue — was taken from freshly killed animals and immediately placed in fixing solution. Both the Larsell pyridine-silver method (Rasmussen, '38) and the activated protargol method (Bodian, '37) were employed in demonstrating nerve fibers. In several specimens on which the Bodian technique was to be used every tenth section was mounted in a separate series and stained with Mallory's connective tissue stain for purposes of orientation. The Larsell method was found

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especially suitable for this study because silver impregnation follows bone decalcification very well. Regardless of the method used, the tissue was sectioned at 10 micra and mounted serially.

OBSERVATIONS

The study of the sphenoid bone in the cat reveals the presence of a well defined cranio-pharyngeal canal in every specimen. It is surrounded by a layer of compact bone embedded in spongy bone and extends from the cranial end of the hypophyseal fossa through the posterior sphenoid bone to the pharyngeal surface of the skull (fig. 1).

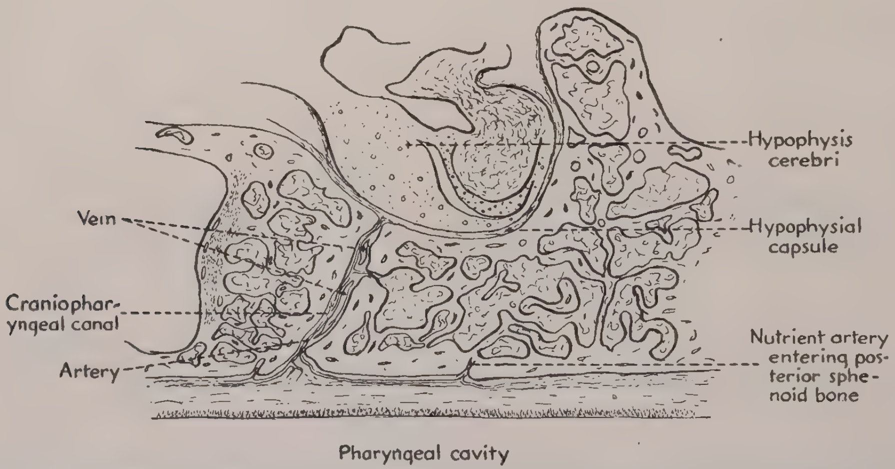

Fig. 1 A composite drawing of the sagittal view of the sphenoid region of the cat. The course of the nerve bundle in the cranio-pharyngeal canal is not represented, but it accompanies the artery and vein.

At the sella entrance a strand of connective tissue, continuous with the hypophyseal capsule, passes through the canal to the pharyngeal surface of the sphenoid bone. Within the canal and along the pharyngeal surface of the bone this connective tissue blends with the periosteum.

The canal contains approximately thirty to forty unmyelinated nerve fibers accompanying a small artery and a relatively larger vein. The majority, if not all, are derived from the internal carotid plexus, having accompanied arteries from the circle of Willis into the hypophyseal capsule. In figure 2 several of these fibers are shown entering the canal and in figure 3 they appear within the canal as a bundle accompanying the blood vessels. At the pharyngeal orifice of the canal, the bundle ramifies in the surrounding tissue (fig. 5). Many of its fibers not only accompany the larger pharyngeal branches of the canal artery (fig. 6), but also pass with nutrient branches into the bony substance. These nutrient arteries, accompanied by nerve fibers derived from the cranio-pharyngeal bundle, enter the bone not only within the canal but also on its inferior surface (figs. 7 and 8). Such nerve fibers can be traced into the spongy substance of the bone, but a short distance after entering the marrow the fibers are lost. This may be due to the difficulty of distinguishing a nerve fiber from reticular fibers impregnated with silver. Figure 9 shows several nerve fibers accompanying an artery into the marrow substance of the sphenoid bone.

In other parts of the bone, nerve fibers much larger than the fibers of the cranio-pharyngeal canal bundle are present (fig. 4). In certain preparations these fibers are large enough to be myelinated and often exhibit the general characteristics of medullated fibers. They can be traced to the endosteum and in general are not as closely related to the blood vessels as the smaller fibers.

DISCUSSION

The close association of the majority of cranio-pharyngeal nerve fibers with the canal artery and its branches strongly suggest that most of them are vasomotor in function. The ramification of some of the fibers in the pharyngeal tissue indicates that some of them may be concerned with secretory functions of the pharyngeal glands.

The presence of myelinated and unmyelinated nerve fibers in Haversian canals and medullary spaces of bone has been described by Stöhr ('28), Rossi ('32), Hurrel ('37) and others. Such nerve fibers as are closely associated with the blood vessels are usually thought to be vasomotor (Stöhr, '28). The function of nerve fibers in medullary spaces is still more speculative. Hurrel ('37) suggests that some of these nerve fibers may be concerned with growth and maintenance of bone. Corbin and Hinsey ('39) believe, however, that such nerves have no specific trophic function.

Recent investigators have pointed out that probably the influence of autonomic nerve fibers upon the circulatory system is not limited to its contractile elements. This has especially been emphasized by Engel ('41) in a study of the influence of autonomic fibers on the transfer of intravenous dyes across synovial membranes. Farber ('40) showed similar autonomic functions in a study of the relationship of vagotomy to acute pulmonary edema. Engel is of the opinion that sympathetic activity increases capillary permeability. If such a hypothesis is correct, the autonomic fibers passing to the bone marrow may be concerned with bone homeostasis as well as with regulation of smooth muscle.

Apparently the majority, if not all, of the fibers contained in the cranio-pharyngeal canal are of autonomic origin, being derived from the internal carotid plexus. If any are derived from the pharyngeal plexus or other pathways, the number must be small. Nevertheless, in experiments involving cranial sympathectomy, the possibility of autonomic fibers entering the cranium via the cranio-pharyngeal canal should be kept in mind.

The larger nerve fibers in the sphenoid bone, which are not continuous with the cranio-pharyngeal nerve bundle, are probably sensory to the endosteum. The origin of these fibers has not been determined. Their size and resemblance to myelinated fibers indicate that they are not of autonomic origin. They cannot be traced into the canal and apparently are not continuous with the nerve fibers contained in the cranio-pharyngeal passage.

SUMMARY

1. A study has been made of the nerve fibers contained within the cranio-pharyngeal canal of eight adult cats.

2. In each case the body of the sphenoid bone was prepared according to the pyridine-silver method used by Larsell and the activated protargol method of Bodian.

3. The cranio-pharyngeal canal of the cat contains a bundle of approximately thirty to forty nerve fibers, a vein, an artery and connective tissue. The majority, if not all, of the nerve fibers found in the canal arise from the internal carotid plexus.

4. Within the cranio-pharyngeal canal the nerve bundle is closely associated with the vascular channels. Its fibers often accompany small branches of the artery into the substance of the sphenoid bone.

5. At the pharyngeal opening of the canal the nerve fibers not only accompany blood vessels into the ventral portion of the sphenoid bone but also ramify in the pharyngeal tissue.

6. Large nerve fibers which are not continuous with the cranio-pharyngeal nerve bundle are also present in the substance of the sphenoid bone.

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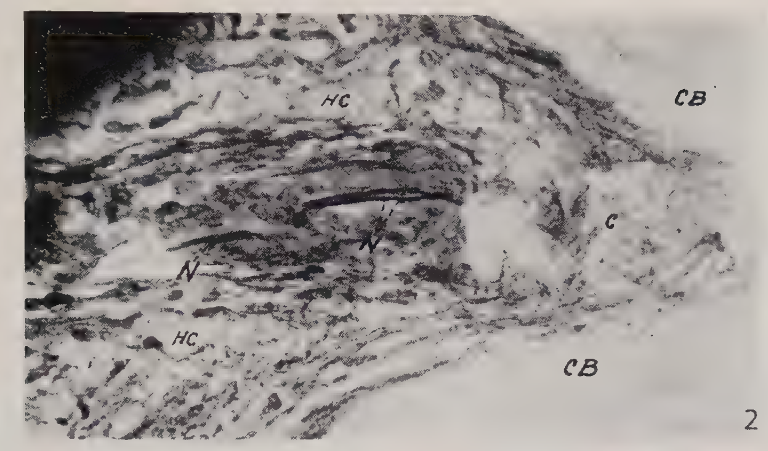
PLATE 1

EXPLANATION OF FIGURES

2 A sagittal section of the hypophyseal region at the entrance of the cranio-pharyngeal canal. The hypophyseal capsule, HC, extends into the cranio-pharyngeal canal, C. Several nerve fibers, N, pass into the canal within the connective tissue. Compact bone, CB, surrounds the canal. (× 310).

3 A frontal section of the sphenoid region showing a portion of the cranio-pharyngeal canal which is approximately equidistant from the hypophyseal and pharyngeal entrances. A bundle of nerve fibers, N, accompanies a vein, V, and an artery, A, through the canal. Surrounding the canal is compact bone, CB. (× 250).

4 Several large fragments of nerve fibers within the sphenoid bone. The fibers, N, are within the spongy bone spaces, SB; but remain close to the compact bone, CB, and endosteum. These nerve fibers are considerably larger than the nerve fibers contained in the cranio-pharyngeal canal. This size difference is evident if the nerve fibers shown in figure 4 are compared with those in figures 7, 8 and 9 which are the same magnification. (× 550).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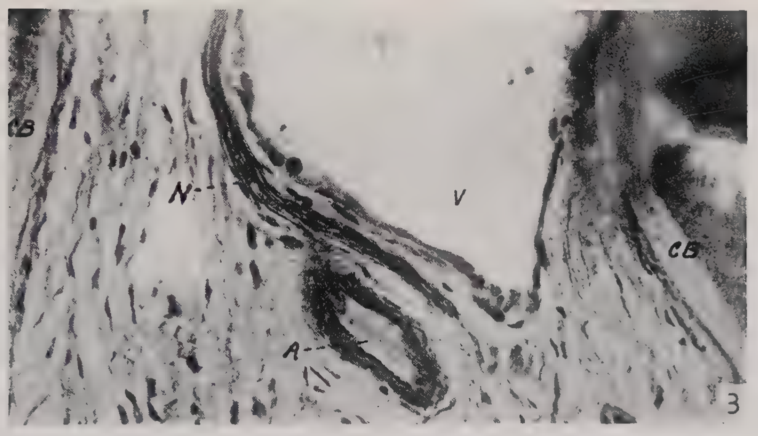
2



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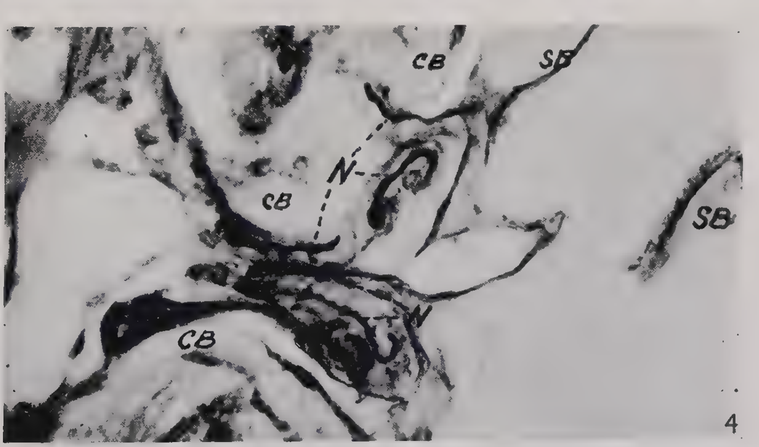
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4

PLATE 2

EXPLANATION OF FIGURES

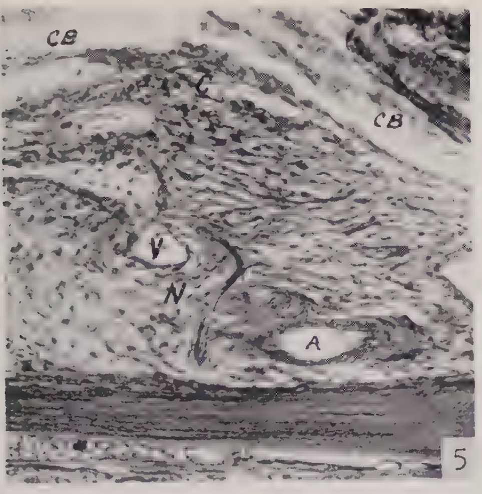
5 A sagittal section slightly lateral to the pharyngeal orifice of the cranio-pharyngeal canal, C. The nerve fibers, N, which are continuous with the nerve bundle contained in the canal, ramify in the adjacent tissue. The artery, A, and vein, V, are coursing posteriorly from the canal opening. Compact bone, CB, surrounds the canal opening. (× 175).

6 A sagittal section of the pharyngeal opening of the cranio-pharyngeal canal, C, surrounded by compact bone, CB. A group of nerve fibers, N, accompanies an artery, A, and a vein, V, (× 90). Inserted in the lower left hand corner, the artery and nerve fibers are shown at a higher magnification. (× 310).

7 A nerve fiber, N, accompanying a small tributary of the cranio-pharyngeal artery, A, into the substance of the sphenoid bone. These structures are within a small nutrient canal, C, surrounded by compact bone, CB. (× 550).

8 A nerve fiber, N, passing with an artery, A, into the pharyngeal surface of the sphenoid bone, (Ph, pharyngeal tissue; CB, compact bone). (× 550).

9 Several nerve fiber fragments, N, associated with an artery, A, within the spongy bone substance, SB. The artery is a tributary of the artery of the cranio-pharyngeal canal and the nerve fibers are continuous with the canal nerve bundle. (× 550).



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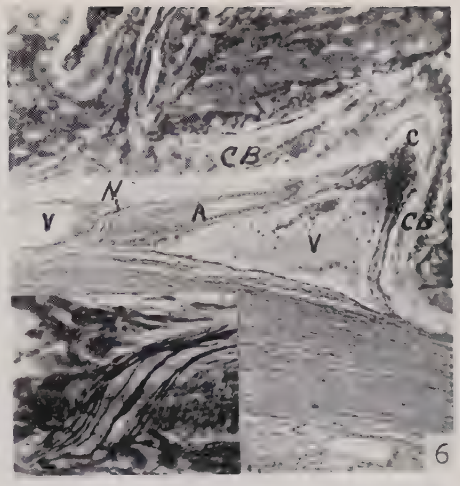

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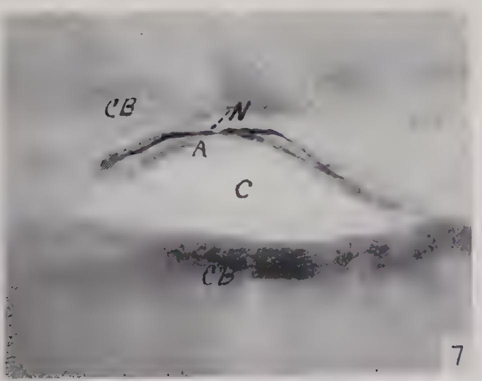

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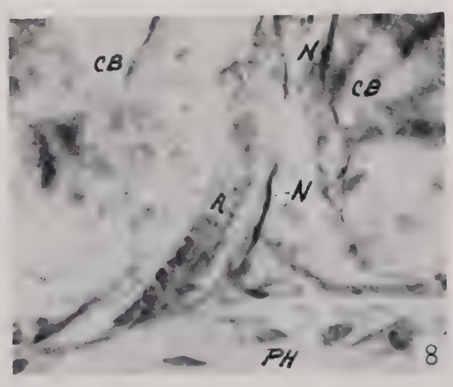

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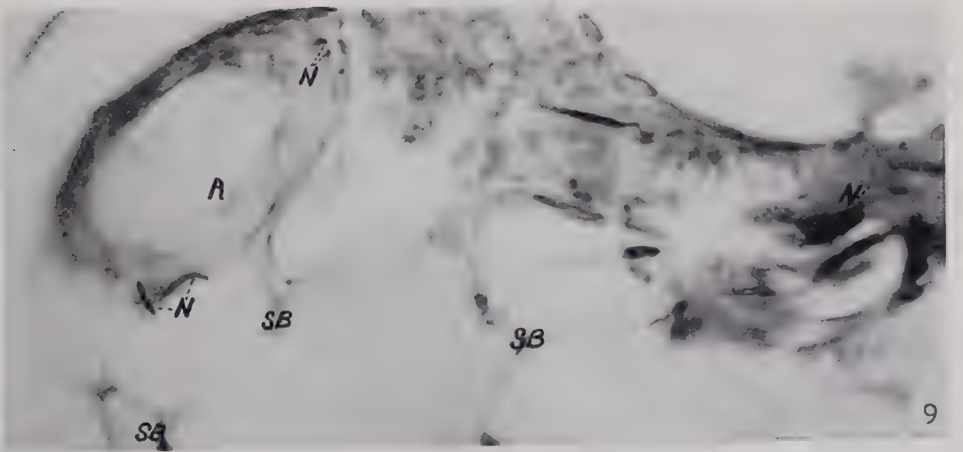
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9

A CYTOLOGICAL STUDY OF THE EFFECT OF TRYPAN BLUE UPON THE LIVER OF THE MOUSE[1]

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ONE PLATE (SIX FIGURES)

Indications of an effect of trypan blue upon the processes of storage and upon the cytology of the liver cell were detected during an attempt to repeat, for the mouse, Glasunow's ('28) finding that in the guinea pig the Golgi net can be outlined with dye granules after repeated small injections. Preparations had been made to demonstrate not only trypan blue granules but also the mitochondria, the Golgi apparatus, bile acids, glycogen and fat. They showed that many cells appear abnormal, that the mitochondria become broader than usual, that glycogen deposition is reduced, and that an accumulation of fat occurs in the liver cells after several injections of the dye. It seemed desirable, therefore, to study a series of animals after a well organized series of injections.

I wish to thank Prof. J. Walter Wilson for his helpful criticism during the course of this investigation.

MATERIAL AND METHODS

A series of young male mice of the Swiss strain were given repeated injections of a solution of vital trypan blue. A 0.5% solution of the dye was made up as directed by Cappell ('29a). At the beginning of treatment, the animals were able to stand intraperitoneal injections of only 0.3 cc. (50 mg. per kilo body weight for a 30-gm. mouse) on alternate days, although later the dose could be increased to 0.5 cc. (83 mg. per kilo). The animals were sacrificed 24 hours after the last injections: all were killed at 5 P.M., and two normal animals of the same age group killed at the same hour of day served as controls. The following table gives the number of injections and length of treatment for each of the animals.

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[1] A portion of a thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Brown University, September, 1943.

[2] Horton-Hallowell Fellow of Wellesley College, 1942–43.

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The same histological methods were adopted as in the previous studies (Deane, '44a, 44b): (1) the Bouin-Delafield method for a study of the general appearance of the tissue; (2) a modified Regaud-Heidenhain method for demonstrating mitochondria; (3) the Champy-Kolatchev method for impregnating the Golgi substance; (4) the Forsgren method for bile acids; (5) the Bauer-Feulgen method for demonstrating glycogen; and (6) a formol-sudan IV method for staining neutral fats. In addition, tissue was fixed in Heidenhain's Susa mixture and the sections counterstained with alum carmine for the demonstration of the trypan blue granules (Pfuhl, '31).

TABLE 1

Data on animals injected with trypan blue.

ANIMAL NO.	AGE IN DAYS	DAYS INJECTED	NO. INJ.
1, a	120	1, 3, 5	3
b	120		
2, a	121	1, 3, 5, 7, 9	5
b	121		
3, a	73	1, 3, 5, 7, 9, 11, 13	7
b	72		
4, a	109	1, 3, 5, 7, 9, 11, 13, 15, 17	9
b	109		
5, a	80	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21	11
b	80		
6, a	112	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25	13
b	113		
7, a	117	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29	15
b	117		
8, a	101	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37 *	18
b	102		
9, a	107	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37,* 39, 41, 43	21
b	108		
10, a	115	1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 37,* 39, 41, 43, 45, 47, 48, 50, 51	26
b	110		

* Size injection before, 0.3 cc., after, 0.5 cc.

Judgments of the quantity of bile acids, of glycogen, and of fat have been made for each animal studied. The symbol (+) indicates that only scattered cells in the lobule contain the substance in question; one or more +'s indicate that an appreciable quantity is present. An attempt has been made to judge the amount of the material in the lobule as a whole, irrespective of whether it is localized in a zone or is distributed evenly in the lobule. It is presumed that these judgments may serve as an approximate measure of the amount of the substance that could be ascertained by biochemical analysis (Deane, 44a).

OBSERVATIONS[4]

The animals became nervous and relatively inactive after about 2 weeks of injections with trypan blue; later they appeared to become somewhat acclimated to the injections and the dose was increased.

In the histological preparations, the Kupffer cells contain great numbers of trypan blue granules in all of the animals, but the liver cells themselves contain dye granules in only three of the animals: no. 9, a and b (twenty-one injections in 43 days, killed on forty-fourth) and no. 10, a (twenty-six injections in 51 days, killed on fifty-second). In these, the blue granules are quite large and round, and only lightly colored. They lie equatorially across the cell (fig. 1).

Not only are there many instances of leucocytic infiltration of the liver in this series, but there are also indications of abnormality in the hepatic cells. In Regaud-Heidenhain preparations, the mitochondria may be dissolved (fig. 3) or the cell may be completely black (fig. 2). Free liver-cell nuclei, with only fragments of cytoplasm attached, sometimes appear in the blood stream. Shrunken cells may be surrounded by leucocytes. In Champy-Kolatchev preparations, isolated cells may be intensely osmiophilic or very light.

Mitotic figures, in all stages, are present in abundance in the liver cells of animals no. 2, a and b (five injections in 9 days, killed on tenth), but in none of the other animals. In several preparations, the nuclei are exceptionally large, and there are examples of nuclei undergoing apparent amitotic division.

The mitochondria, in the control animals, appear as long heavy rods in the peripheral zone, filaments in the middle zone, and still finer and more tangled filaments in the central zone. In the experimental series, those of the peripheral zone are generally short, often swollen and sometimes even irregular in form. In the middle zone, the chondriosomes are always more elongate than in the peripheral zone and are extremely crowded. In the central zone, they are always very fine and elongated filaments. They become progressively sparser and lighter in their staining reaction toward the central vein, and are even fragmented in this region in pairs no. 4 to 8 (nine to eighteen injections).

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[4] Detailed observations are included in the thesis which is available at the John Hay Library, Brown University.

The Golgi substance in the hepatic cells of the control animals is in the form of a broken network, lying equatorially across the cell, surrounding the nucleus and reaching to the bile capillaries. The strands of the network are broadest in the cells of the peripheral zone. In all of the preparations of the experimental series, also, the Golgi apparatus occupies this position in the cell (figs. 4 and 5). No Golgi network is discernible in the cells near the veins in the preparations of pairs no. 7 through 9 (fifteen to twenty-one injections). The Golgi strands are particularly large in the preparations of pair no. 10.

The amounts of bile acids, of glycogen, and of fat are recorded in table 2.

TABLE 2

Bile acid, glycogen, and fat content of liver in histological preparations.

NUMBER OF ANIMAL	INJ.	BILE ACIDS	GLYCOGEN	FAT
Cont., a	0	+++++++	+	0
b	0	+++++++	0	0
1, a	3	++++++++	(+)	0
b	3	++++++++	(+)	0
2, a	5	++++++++++	(+)	0
b	5	++++++++	(+)	0
3, a	7	+++++++++	(+)	0
b	7	+++++++++	0	++
4, a	9	++++++++++	0	0
b	9	++++++++	0	0
5, a	11	+++++++++	++	0
b	11	++++++++++	+	+
6, a	13	+++++++++	+++	+++
b	13	++++++++	(+)	+++++
7, a	15	++++++++++	+++	++
b	15	++++++++	++	+++++
8, a	18	+++++++	+	+(+)
b	18	+++++++++	0	++
9, a	21	++++++++++	++	++
b	21	++++++++++	0	+++++
10, a	26	....	0	0
b	26	....	+	0

Granules of bile acid material are crowded in all of the livers throughout the liver lobule. If anything, they are more numerous than in non-injected animals.

The quantity of glycogen is very low in the animals at the beginning of the series (three to nine injections), but later it is present in at least one of the livers of each pair. When present, it is generally more concentrated in the central part of the lobule, although it is sometimes distributed irregularly in the lobule, and in pair no. 7 (fifteen injections in 29 days, killed on thirtieth) it is most concentrated in the peripheral region.

Fat is present in the livers of no. 3, b (seven injections in 13 days, killed on fourteenth), no. 5, b (eleven injections in 21 days, killed on twenty-second), pairs no. 6 through 9 (thirteen to twenty-one injections), but virtually absent in pair no. 10 (twenty-six injections in 51 days, killed on fifty-second). In animals no. 3, b, 5, b, and pairs 6 and 7, the fat is more concentrated in the central part of the lobule (fig. 6); in pair no. 18 it is evenly distributed in the lobule, and in pair no. 9 it is more concentrated in the middle and peripheral zones than in the central. The fat exists as crescent-shaped or irregular droplets that are quite crowded in the cell.

DISCUSSION

The mouse can tolerate repeated injections of only 50 mg. per kilo of the vital dye, trypan blue, on alternate days. With larger injections, the animals become sick and die in a few days. After repeated injections of the dye solution, granules are stored in great quantity by the Kupffer cells in the liver but only rarely by the hepatic cells themselves. Even though there is no visible segregation of trypan blue by the parenchymal cells except in a few instances, there is every evidence that the liver cells are adversely affected by the dye in some way. There are many examples of necrotic changes and apparent death of the liver cells — indications of phagocytosis, fragmented liver cells in the blood stream, black and light cells in both the mitochondrial and the Golgi substance preparations.

The nature of the toxicity of trypan blue, either to the animal as a whole or to the liver cells, is unknown. It has been reported that large doses are fatal, and so are repeated doses, unless very small (Cappell, '29a). Anderson, Emerson and Fisher ('34) found that some mice are killed by one intraperitoneal injection of 100 mg. of the dye per kilo, and all mice by 400 mg. per kilo. Animals that died gave evidence of congestion of the lungs. The authors reported that their animals showed symptoms of a depression of the central nervous system with repeated injections, and my animals too became inactive after about 2 weeks of treatment. It is characteristic of the colloidal acid dyes that they are taken up by the reticulo-endothelial system, and it has been suggested that when this system is saturated with such a foreign substance its activities become ''blocked.'' Whether such a ''blockade'' is ever responsible for the death of an animal, however, is still open to question (Maximow, '32, p. 760; Jaffé, '38, p. 1052).

As for the liver cells themselves, it does not appear that the effect of the trypan blue, if it is a direct one, is due to its penetration into the cell. In spite of the many evidences of cell death, the dye is visible within the cells in only a few of the animals. Cappell ('29b) has previously reported that it was virtually impossible to cause the segregation of trypan blue by the hepatic cells of the mouse, although it is readily segregated in the rabbit and the guinea pig.

An interesting phenomenon in this series is the occurrence of mitosis in the liver cells of pair no. 2 (five injections in 9 days, killed on tenth). Pfuhl ('38) has previously reported that he found mitotic figures in the livers of guinea pigs killed 6 and 8 days after one injection of trypan blue. He saw no evidence of cellular degeneration and believed the mitotis of the liver cells to be caused by strain placed upon the organ by the necessity for excreting the dye, i.e., the organ is enlarging to meet the new functional demands. He also reported an increase in the size of the nuclei (indicating polyploidy) and in the number of binucleate cells. In my series I have noted the same effects, and in some preparations there are nuclei apparently undergoing amitosis, which is probably the mechanism for the formation of binucleates (Beams and King, '42). Pfuhl's explanation of the cause of the mitoses seems improbable in view of the fact that the mouse liver does not appear to excrete trypan blue as much as the guinea pig liver does. If the effect of the trypan blue on the liver cells is a direct one, it must act in very minute quantities.

From the results of the preliminary experiment it was believed that trypan blue reduces the storage of glycogen. In the final series, this appears to be the case in pairs no. 1 through 4 (three to nine injections), since none of the preparations contain any glycogen at all. After this number of injections, at least one of the livers of the pair contains as much glycogen as the control livers. Unfortunately for a study of this particular function of the liver, these animals were killed at 5 P.M. which is the time of almost minimal storage of glycogen. If it proves true, however, that trypan blue does inhibit the storage of glycogen during the time when the animals are becoming accomodated to the dye, we have as yet no explanation for it.

Fat is present in the livers of pairs no. 6 through 9 (thirteen to twenty-one injections). It is located first in the center of the lobule, then distributed evenly and is finally most concentrated in the peripheral part of the lobule. It has disappeared entirely in pair no. 10 (twenty-six injections). The cause of this progressive change is completely unknown. The fatty condition might be due to a fatty degeneration of the cells, in which the lipoproteins of the protoplasm itself are broken down (Macheboeuf, '36). This could result from the direct toxic action of the dye and would occur first in the central region because of the relative anoxia of this zone. Or the fatty condition might have resulted from fatty infiltration of the liver. This occurs when there is some upset in the metabolic balance of the body (Bloor, '39). Why it should occur with trypan blue injections, however, is not apparent.

In the control animals killed at 5 P.M. the liver cells are full of bile acid granules, which is to be expected since the late afternoon has been shown to be the time of maximal secretion of the bile acids (Deane, '44a). In the animals of the experimental series, too, there are a large number of granules in the cells, possibly even more than usual.

In all of the livers of animals injected with trypan blue the mitochondria of the peripheral zone are markedly shorter and often broader than in the control animals. The significance of this condition is obscure, although it was suggested earlier (Deane, '44a) that a rounding of the mitochondria indicates an augmented activity of the cells. In the case of trypan blue injections, the rounding of the mitochondria may reflect the "strain" placed on the organ by the presence of a toxic substance, and it may be related to the many other evidences of pathological reaction. Moribund changes, after all, are only accentuations of normal physiological changes, and the line of demarcation between the two is never sharp.

In the central zone, the mitochondria of pairs no. 4 through 8 (nine to eighteen injections) are much sparser, lighter and more fragmented than in the control livers. Perhaps this change is somehow related to the fact that fat is present in this zone in the preparations of pairs no. 5 through 8 (eleven to eighteen injections). In the animals of the diurnal cycle series in which fat was present, a similar change occurred in the mitochondria of the central cells (Deane, '44a). The location of the fat and the loss of siderophily by the mitochondria may be due to a common cause, possibly an increased anoxia in the central zone of the lobule.

Chondriolysis is prevalent in isolated cells (fig. 3). Other cells are entirely black (fig. 2). As in animals on a high-sugar diet (Deane, '42) and in newborn animals (Deane, '44b), such phenomena probably indicate cell strain and possibly cell death. In these preparations, phagocytosis and the fragmentation of cells indicate that cell death does actually occur in some instances.

The Golgi apparatus, both in controls and experimentals, lies equatorially across the cell in loose, broken networks — along the bile capillaries and surrounding the nucleus (figs. 4 and 5). This corroborates the description of Nassonov ('26). Since the trypan blue granules, when found, have a similar distribution in the cell they are probably associated with the Golgi substance. Many previous workers have reported that the Golgi material is involved in the segregation of the dye (Nassonov, '26; Ludford, '28; Glasunow, '28; Makarov, '31; and Pfuhl and Dienstbach, '38). Nevertheless there is no apparent change in the Golgi apparatus associated with the visible segregation of the dye. In the no. 9 preparations, the Golgi strands are fine and not intensely osimiophilic, whereas in the no. 10 preparations, the strands are very large indeed (fig. 5).

In the livers of pairs no. 7 through 9 (fifteen to twenty-one injections) there is no Golgi substance apparent at all in the zones of cells around the portal and central veins. This may indicate a real abnormality in the tissue since the Golgi material is known to disappear in dying cells (Kirkman and Severinghaus, '38).

In the preparations from animals with many injections of the dye, there is a great deal of variation in the osimophily of the cytoplasm itself. Some cells are very light, others are very dark and some completely opaque. As yet there is very little knowledge as to the significance of osimophily of the cytoplasm in gland cells. De Robertis ('41) reports that in the parathyroid, the osmiophilic cells are actively secreting; on the other hand, Severinghaus ('33) believes dark cells in the thyroid are degenerating. In the liver, the presence of so many cells of such variable osmiophily may indicate an abnormality of some kind, if only hyperactivity.

This study was undertaken as part of a series in which the relations between changes in the cell organelles and the processes of storage and secretion were being studied. As in the previous reports (Deane, '44a), '44b), there appears to be no direct activity of either the mitochondria or the Golgi apparatus in the processes of storage of glycogen or fat, or of secretion of bile acids.

SUMMARY AND CONCLUSIONS

1. Mice were given small injections of trypan blue on alternate days and were then sacrificed after progressively longer treatment. Mitochondrial, Golgi apparatus, bile acid, glycogen, and fat preparations of the livers were studied.

2. Trypan blue is present in the liver cells of only those animals injected at least twenty-one times. After much shorter treatment, however, it appears to be toxic to the animal and even to the liver cells. It also causes mitosis of liver cells in those animals injected five times.

3. With the injection of the dye there is a reduction in the amount of glycogen storage for the first 2 weeks.

4. Fat is present in the livers of those animals injected thirteen to twenty-one times. It shows a progressive change in its distribution.

5. The secretion of bile acids appears not to be affected by the injection of trypan blue.

6. The mitochondria of the peripheral zone are shorter and broader than normal in the injected animals. Moreover there are light and dark cells in many of the preparations, suggesting cell abnormality.

7. The Golgi substance undergoes no visible change in relation to the segregation of trypan blue. There are signs of cellular abnormality in the preparations for the Golgi substance also.

8. There is no indication of any relationship between the changes in the mitochondria and the Golgi substance and those in the processes of storage and secretion.

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PLATE 1

EXPLANATION OF FIGURES

The photographs were taken using compensating oculars and a 2-mm. oil immersion (90 ×) apochromat objective. The picture of the Susa preparation (for trypan blue) and that of the sudan IV preparation (for fat) were taken with a Wratten X_1 green filter. Those of Heidenhain iron-alum hematoxylin preparations (for mitochondria) and of Champy-Kolatchev preparations (for the Golgi substance) were taken without any filter.

1 Animal in preliminary experiment; twenty-three injections in 51 days, killed on fifty-second. Susa preparation. Trypan blue granules lie equatorially across liver cells. Magnification, × 1350.

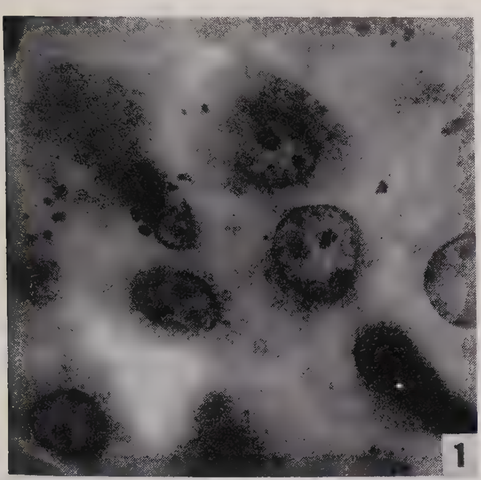
2 Animal no. 4, a; nine injections in 17 days, killed on eighteenth. Regaud-Heidenhain preparation. Middle zone. Mitochondrial filaments crowded and beaded. Black cell in middle apparently undergoing phagocytosis. Magnification, × 1350.

3 Animal no. 9, b; twenty-one injections in 43 days, killed on forty-fourth. Regaud-Heidenhain preparation. Mitochondria crowded, fine, beaded filaments. Siderophilic clumps in the cytoplasm. One cell undergoing chondriolysis. Note large Kupffer cells. Magnification, × 1350.

4 Animal no. 2, b; five injections in 9 days, killed on tenth. Champy-Kolatchev preparation. Golgi substance consists of fine strands near nucleus, in intermediate cytoplasm, and along bile capillaries. Magnification, × 1350.

5 Animal no. 10, b; twenty-six injections in 51 days, killed on fifty-second. Champy-Kolatchev preparation. Very large Golgi strands lying between nuclei and bile capillaries. Magnification, × 1350.

6 Animal no. 6, a; thirteen injections in 25 days, killed on twenty-sixth. Sudan IV preparation. Central zone: cells filled with fat droplets. Magnification, × 1250.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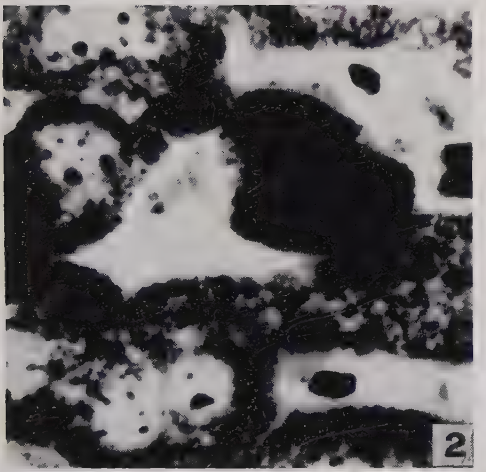
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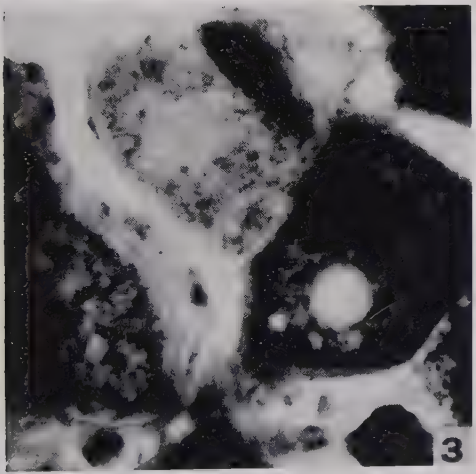
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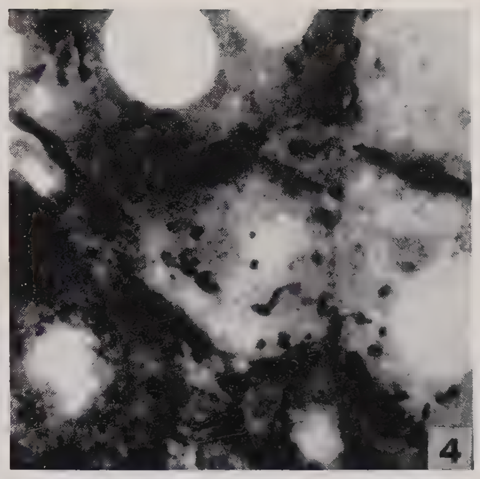
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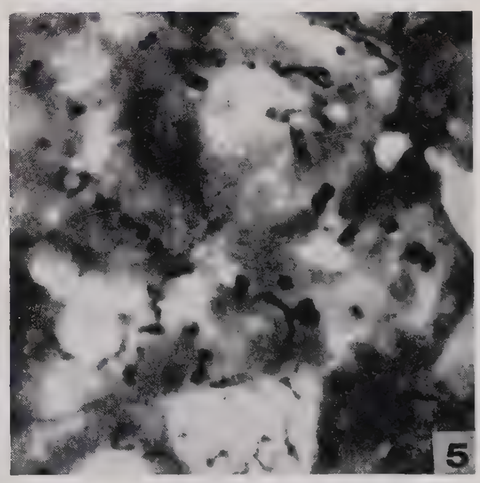
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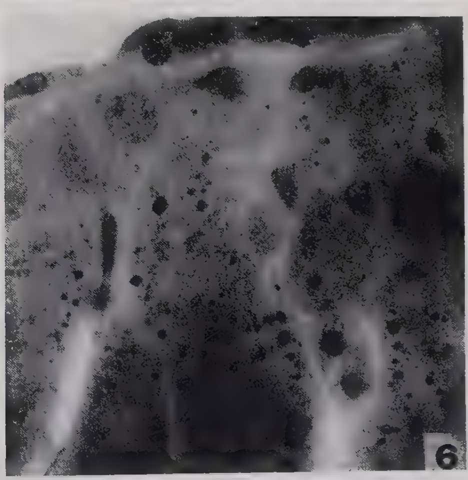
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THE HISTOLOGY OF THE MENINGES OF THE TOAD (BUFO)

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FOUR FIGURES

INTRODUCTION

Since Key and Retzius (1875) published their fundamental monograph, many authors have adequately described the meninges of man and other mammals. ''But,'' as Weed wrote in 1932, ''it is amazing how little is actually known of the details of the anatomy of the meninges in the lower vertebrates.'' The investigators who have been interested in the comparative anatomy of the meninges (for bibliography see Kappers, Huber, and Crosby, '36) have concerned themselves more with the homologies and with the embryologic and phylogenetic development of these membranes than with their histology.

Yet the meninges of the lower vertebrates deserve closer study, because, at least, in certain fishes and amphibians, they contain structures which are of general histological interest. Such structures as the meningeal myeloid tissue overlying the fourth ventricle of the ganoids (Scharrer, '44), or the peculiar inclusion-bearing cells covering the arachnoid membrane of the toad are not encountered in the more completely differentiated meninges of mammals. Evidently, the meninges of the lower vertebrates should be considered not simply as stratified connective tissue protecting the central nervous system, but as immature connective tissue still bearing mesenchymal potentialities which in one respect or another may be full expressed in certain fishes, amphibians, or reptiles.

The present study concerning the meninges of the toad was undertaken as a contribution to the comparative histology of the meninges. Special attention was paid to structures and cell-types that are peculiar to the meninges of amphibians, particularly the genus Bufo.

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MATERIALS AND METHODS

In this investigation the meninges of Bufo cognatus, B. woodhousei, and B. terrestris were studied. Material for comparison was obtained from the catfish (Noturus flavus), the turtle (Graptemys geographica), the snake (Natrix sipedon), and the dog.

Almost all of the toads were adult females with an average weight of 61 gm. Until the time of fixation they were housed in large wooden crates, 36 inches long, 24 inches wide, and 16 inches deep. A 6-inch layer of loose earth covered the floor. The toads were fed live earthworms and insects, and water was provided in a large basin set in the earth. Such terraria can accommodate up to 2 dozen toads without crowding and with relatively natural environment and food.

Seven of the toads were subjected to intracranial injections of foreign particulate matter. India ink (approximately 1% solution made up in 0.65% saline) was injected intracranially into three toads through a gauge 27 needle inserted just above the external nares and directed dorsally and posteriorly toward the midline. After the needle had entered the cranial cavity, it was possible to inject about 0.01 cc. of the solution without resistance. In one of these toads the solution passed into the subarachnoid space, and in two it passed into the subdural space. The animals were killed at the end of 36 hours, 65 hours, and 5 days after the injections had been made. In four toads intraperitoneal hemorrhage was produced through an abdominal incision by cutting small blood vessels to the viscera. Some of this blood was collected and injected into the cranial cavities of the same animals. These toads were killed 6, 10, 14, and 18 days after the operation and injection. During all these procedures the toads were narcotized with cracked ice.

Ten specimens, operated and normal, were fixed by perfusion through the heart with Zenker's fluid followed by Zenker-formol. The brains were dissected out with the meninges intact and were immersed in the fixative for about 6 hours. Liver, kidney, spleen, gonads, and intra-abdominal blood clots were also preserved by the same method. The tissues were embedded in paraffin and cut 5 to 6 μ thick. Serial sections were stained with Foot's modification of Masson's trichrome. Selected sections were stained with haematoxylin-eosin-azure, with Heidenhain's azan for collagen, with the Foot-Bielschowsky silver method for reticulum, and with Verhoeff's stain for elastic tissue. Other sections were treated with Feulgen's stain for thymonucleic acid and with the Prussian blue reaction for iron-containing substances.

The brains and meninges of four normal toads were fixed in 10% formalin and embedded in paraffin. Sections were treated with the benzidine test as modified by Pfaff and Williams ('42). Supravital preparations of the arachnoid were made from several toads. Total spreads of arachnoid and dura were treated with 1% silver nitrate in an attempt to demonstrate the mesothelium lining these membranes. Strips of pericardium treated simultaneously with the same solution were used as controls.

For the study of the topographical relationships of the meninges, the head and spinal column of one toad were fixed by perfusion with Zenker-formol, decalcified with 3.5% aqueous trichloracetic acid, and embedded in nitrocellulose. Serial sections, 10 and 20μ thick, were stained with Foot's modification of Masson's trichrome and with Heidenhain's azan.

OBSERVATIONS

In the following account of the meninges of the toad, the identification of the membranes surrounding the brain and spinal cord is based upon the studies of Flexner ('29), according to whom the development of the meninges in amphibia is essentially the same as that in mammals (Weed, '17). The meninges of the toad (fig. 1) consist of three membranes, dura, arachnoid, and pia, separated by fluid-containing spaces.

The pachymeninx. The dura is a tough fibrous membrane adherent to the inner walls of the skull and obviously identical in position and function with the internal periosteum. It is composed of laminae of heavy collagenous fibers between which lie the nuclei of fibroblasts. The fibers in each lamina lie parallel to one another and pass obliquely across those in the laminae above and below it. On the inner surface of the dura the fibroblasts are elongated and flattened, producing in cross sections the appearance of a lining membrane. Reduced silver preparations, however, fail to demonstrate any mesothelium lining the subdural space of toads.

The leptomeninges. The arachnoid completely surrounds the brain, from which it is separated by the subarachnoid space. On the dorsal surface of the brain it dips into and joins the chorioid roof of the third ventricle, forming a depression in which the epiphysis and the paraphysis lie (fig. 2). Posteriorly over the fourth ventricle, the membrane stretches across the chorioid plexus and is continuous with the spinal arachnoid. Ventrally, it does not enclose the hypophysis, which lies in the subdural space. Laterally, the arachnoid ensheathes the cranial and spinal nerves, joining with the dura and the perineurium in the foramina. These relationships are diagrammatically represented in figure 1.

Blood vessels and melanophores are almost completely absent from the perfectly transparent arachnoid membrane. The vessels which one sees in the living preparation lie in the subarachnoid space and in the pia. As is indicated in figure 3A, the arachnoid membrane is composed of four to seven thin, almost acellular, hyaline lamellae. The innermost layer consists of interrupted strands of fine collagenous and elastic fibers which are continuous with similar fibers in the subarachnoid trabeculae. The outermost lamina consists of a discontinuous network of argyrophil fibers. The intermediate layers are composed of a glassy, refractile substance which stains with varying intensities of red in acid fuchsin (Masson's trichrome stain) but which does not take the stains for collagen or for elastin. With the Foot-Bielschowsky

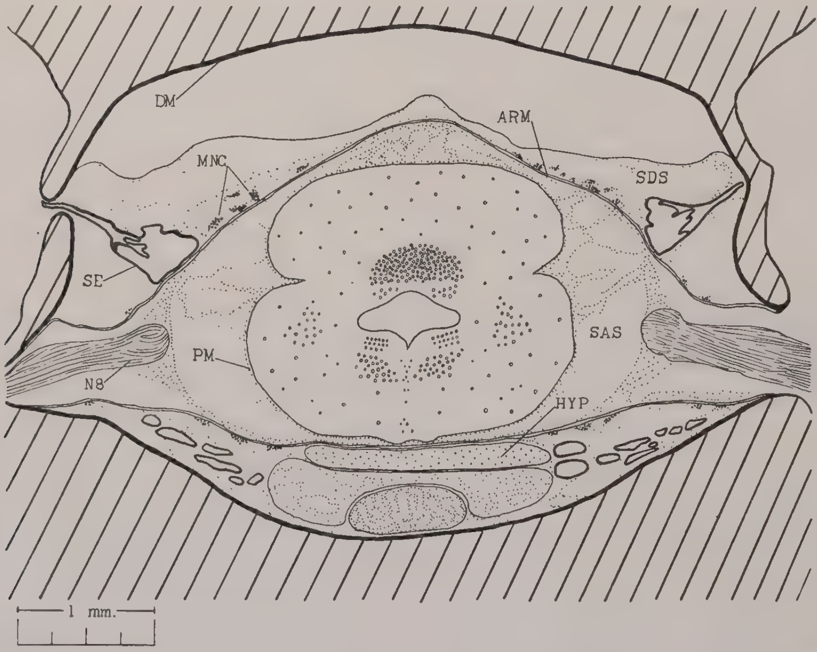

Fig. 1 Semi-diagrammatic drawing of a transverse section through the brain stem and surrounding structures of the toad Bufo cognatus. The section shows the relationships between the arachnoid membrane and the cranial nerves, hypophysis, and saccus endolymphaticus. Bone is indicated by diagonal ruling. ARM, arachnoid membrane; DM, dura mater; HYP, hypophysis; MNC, meningocytes; N8, eighth cranial nerve; PM, pia mater; SAS, subarachnoid space; SDS, subdural space; SE, saccus endolymphaticus. Fix., Zenker-formol perfusion, nitrocellulose, 20 μ, Foot's modification of Masson's trichrome.

reticulum stain the intermediate layers are very lightly and imperfectly impregnated with silver. The arachnoid membrane appears, therefore, to be composed of very immature collagen disposed in lamellae incompletely covered on both inner and outer surfaces by discontinuous membranes of more mature collagen.

Fibroblast-like cells line the undersurface of the arachnoid. The large vesicular nuclei of these cells have thin, irregular outlines and finely granular, dustlike chromatin; usually there is a faintly acidophil nucleolus in each nucleus. The nuclei often lie in pairs with their indented surfaces facing each other. Preparations stained with Heidenhain's azan show fine fibers in the vacuolated cytoplasm of these cells.

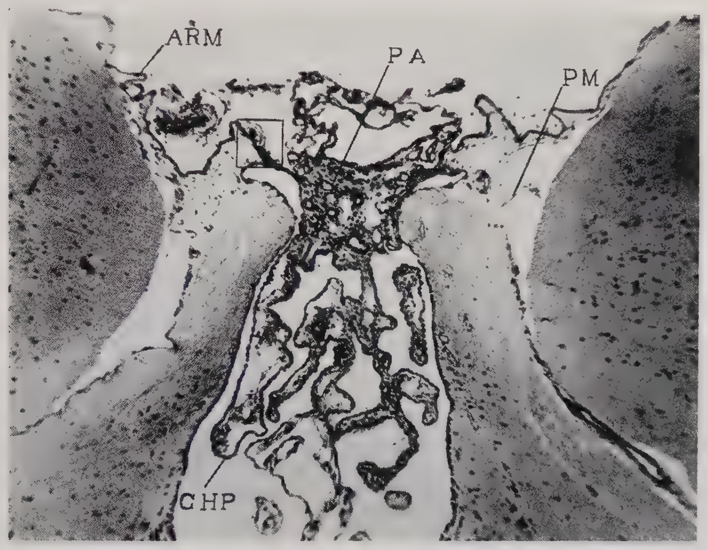

Fig. 2 Transverse section through the third ventricle of Bufo cognatus, showing the relationship between the arachnoid membrane and the chorioid plexus. The area within the square to the left of the paraphysis is drawn at a higher magnification in figure 3A. ARM, arachnoid membrane; CHP, chorioid plexus; PA, paraphysis; PM, pia mater. Fix., Zenker-formol perfusion, paraffin, 6μ, Foot's modification of Masson's trichrome. Photomicrograph, × 45.

The pia is a very delicate, thin, collagenous membrane tightly adherent to the nervous tissue and covered by sparsely distributed fibroblast-like cells similar to those just described. In contrast to the arachnoid, the pia contains numerous small blood vessels. Following the branches of these vessels, the pia dips into the brain substance to form the lining of the perivascular spaces. Large melanophores are scattered over the surface of the pia, most commonly over the optic lobes where the pigmented cells surround blood vessels.

The subarachnoid space. Between the arachnoid membrane and the pia lies the subarachnoid space, occupied by a loose meshwork of stellate cells the attenuated processes of which constitute the arachnoid trabeculae. These cell processes clothe a fragile network of fine collagenous fibers which connects the pia with the arachnoid membrane. Interspersed among the trabeculae are a few lymphocytes and polymor-

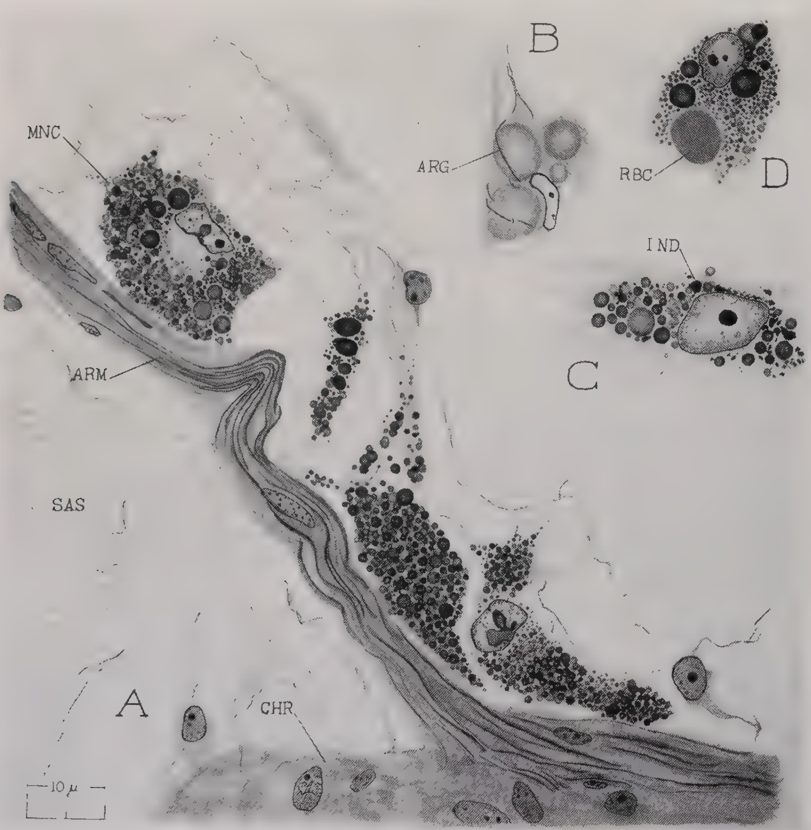

Fig. 3 Camera lucida drawings of meningocytes from Bufo cognatus. A, Detail from the squared area in figure 2, showing three inclusion-laden meningocytes on the external surface of the laminated arachnoid membrane. ARM, arachnoid membrane; CHR, chorioid roof of the third ventricle; MNC, meningocyte; SAS, subarachnoid space. B, Meningocyte stained according to the Foot-Bielschowsky method for reticulum. ARG, argyrophil fiber. C, Meningocyte from the arachnoid membrane over the olfactory nerves of a toad injected intra-cranially with India ink 65 hours before fixation. IND, ingested India ink. D, Meningocyte containing the remnant of an ingested erythrocyte (RBC), from the arachnoid of a toad injected with autogenous blood 14 days before fixation. The tissues were fixed by perfusion with Zenker-formol, embedded in paraffin, sectioned 5 to 6μ thick, and all, except the cell pictured in figure B, stained with Foot's modification of Masson's trichrome.

phonuclear leucocytes. In some specimens these free cells are numerous and contain blood pigment. The subarachnoid space is best developed in its cranial portion and decreases in width rather abruptly over the posterior portion of the medulla. Around the spinal cord the space is only a thin crevice between the pia and the arachnoid (fig. 4).

The subdural space. In the toad, dura and arachnoid are separated by a wide cavity which is occupied principally by the elaborately developed saccus endolymphaticus. The relationships and morphology of this organ have been fully described by Dempster ('30) and need not be further discussed here. The saccus endolymphaticus divides the subdural space into two smaller cavities. At intervals stellate cells extend across the external cavity and suspend the saccus from the dura. The inner cavity, between the arachnoid and the saccus, contains specific cells which will now be described.

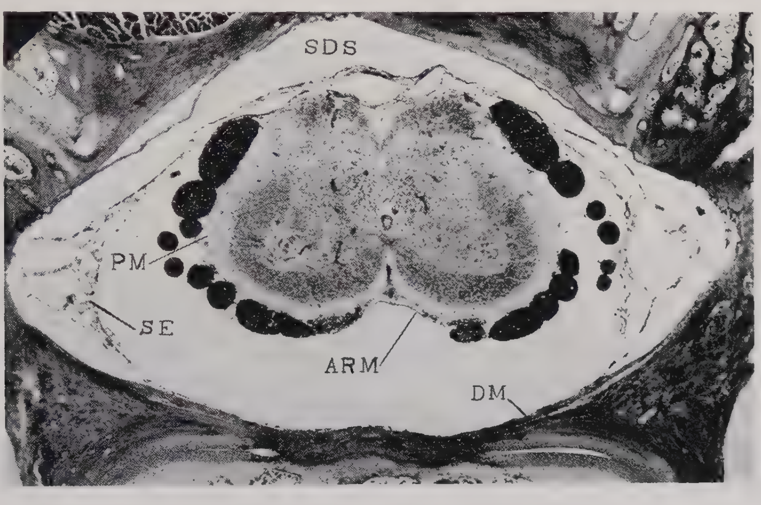

Fig. 4 Transverse section through the spinal cord of Bufo cognatus, surrounded by spinal nerves and meninges. The subarachnoid space is very narrow. In several places the dura has separated from the inner walls of the vertebral canal. ARM, arachnoid membrane; DM, dura mater; PM, pia mater; SDS, subdural space; SE, saccus endolymphaticus. Fix., Zenker-formol perfusion, nitrocellulose, 10μ, Foot's modification of Masson's trichrome. Photomicrograph, × 29.

The external surface of the arachnoid membrane is bare except for a few flattened fibroblasts and the irregularly distributed characteristic cells which were first described by Scharrer ('35). These peculiar cells are large stellate elements which typically contain clusters of acidophil cytoplasmic inclusions (fig. 3). As the following description will disclose, these cells are peculiar to the meninges of certain amphibians. Scharrer suggested that they might correspond to the macrophages in mammalian meninges which Cushing ('26) designated as "meningocytes." For the sake of brevity I shall borrow the term from Cushing and refer to the inclusion-bearing cells in amphibian meninges as meningocytes even though they do not correspond precisely in situation or morphology to the mammalian meningocytes.

The meningocytes in toads may be found anywhere along the outer surface of the arachnoid membrane, most commonly on the ventral aspect of the brain. They tend to collect in folds and corners, for example, along the olfactory nerves, and along the ventral surface of the hypothalamus. Another favorite place for them is on the slopes of the depression of the arachnoid over the chorioid plexus of the third ventricle (figs. 2 and 3A). Here they may form in some specimens a layer two or three cells deep.

The total number of these cells varies considerably from specimen to specimen. In some they are difficult to find; in others they are numerous in all areas of the cranial arachnoid. In all specimens the number of meningocytes decreases posterior to the cerebellum, and although they may occur in the connective tissue of the chorioid plexus of the fourth ventricle, they are absent from the meninges of the spinal cord. These cells appear in the meninges of all three species examined, B. cognatus, B. woodhousei, and B. terrestris. Scharrer found them in B. vulgaris and in Salamandra maculosa.

The nuclear pattern of the meningocyte is characteristic. The nucleus is generally in the center of the cell or slightly to one side. Within the irregular, deeply staining nuclear membrane lie several small particles of chromatin and a single, slightly acidophil nucleolus. Often minute particles of chromatin are arranged along the membrane, leaving the central portion of the nucleus clear.

The faintly staining, vacuolated cytoplasm of the meningocyte is usually closely attached to the arachnoid membrane. But in certain regions, especially above the olfactory nerves and ventral to the hypothalamus, the cells are spread out in the subdural space, their long thin processes linking one cell with another and with the arachnoid. At the base of the meningocytes and in the distal ends of the processes lie a few thin argyrophil fibers (fig. 3B).

Most of the meningocytes contain collections of brilliantly acidophil inclusions, ordinarily packed closely one upon another, as shown in figure 3A. In many cells the inclusions are massive; a single, large, angular mass may occupy most of the cell. If the granules in a cell are numerous they are usually small and equal in diameter, but if the granules are few in number they tend to be of various sizes. In B. cognatus and B. woodhousei the granules are spherical, whereas, in B. terrestris they are rod-shaped and flattened.

Fixation in Zenker-formol is required for the best demonstration of the meningocyte granules. After fixation in 10% formalin, they take the acid stains very faintly or not at all, and they may easily be overlooked unless searched for under subdued light. The granules stain most brilliantly with the red component of Masson's trichrome stain. In haematoxylin-eosin-azure the granules are pale lavender. In supra-vital preparations the granules stain rapidly with neutral red and remain discrete for many hours, even after the cell has died.

The meningocyte is unlike any cell in amphibian blood (Freidsohn, '10). It is easily distinguished from the eosinophils of the blood and connective tissues by its nuclear pattern, large size, and characteristic granules. A thorough search of the liver, kidneys, intestinal tract, and subcutaneous connective tissue fails to reveal any cells resembling it.

In Salamandra maculosa and in toads in which intracranial hemorrhage had been induced, Scharrer ('35) found that the larger inclusions of the meningocytes contained basophil material or nuclei. This finding led him to conclude that all the inclusions were derived from ingested erythrocytes. In my preparations of normal meninges inclusions suggestive of freshly ingested erythrocytes could not be found. Many of the large inclusions did contain small basophil masses, but Feulgen's stain failed to demonstrate any nuclear material in them. As indicated by the benzidine test and the Prussian blue reaction, cytoplasmic constituents of erythrocytes are likewise absent from the inclusions. Furthermore, no cells resembling meningocytes could be found in the intra-abdominal hemorrhages which were produced by cutting small blood vessels, although phagocytosis of extravasated red cells by macrophages had progressed actively. The characteristic inclusions of meningocytes, therefore, cannot be regarded as partially digested erythrocytes. This statement, however, does not mean that the meningocytes may not ingest erythrocytes under experimental conditions, for when blood is injected into the subdural space a few meningocytes will phagocytose the foreign cells.

Reaction to foreign particulate matter. Injections of autogenous blood or of diluted India ink into the meningeal spaces of the toad elicited an intense inflammatory reaction characterized by a serous exudate. Polymorphonuclear leucocytes were the predominating cells, but eosinophils and lymphocytes were also common. If the foreign substances were injected into the subarachnoid space no inflammation occurred in the subdural space, and if the injection was made solely into the subdural space the subarachnoid space remained undisturbed. Thus, the arachnoid serves as a competent limiting membrane.

In this study particular attention was paid to the reactions of the meningocytes to injected foreign matter. Whereas the cells of the exudate rapidly engulfed considerable amounts of carbon or blood, the meningocytes phagocytosed little or none of the injected matter. After 65 hours following the injection of India ink, only the meningocytes in the olfactory region, at the site of injection, consistently contained amounts of carbon comparable to those in the leucocytes (fig. 3C). The remaining meningocytes, distributed over the arachnoid, contained few or no carbon particles even though they were adjacent to leucocytes crowded with ingested India ink. The reaction of the meningocytes after 5 days was no more intense than that after 65 hours, but mitotic figures had begun to appear in the meningocytes over the dorsal surface of the optic lobes. In toads injected subdurally with autogenous blood, the meningocytes which had ingested erythrocytes or their products (fig. 3D) were rare, even after 18 days following injection. In contrast, the cells of the exudate contained great numbers of erythrocytes and pigment bodies.

DISCUSSION

Homology. Ever since Sterzi ('00–'01) reported his extensive studies on the comparative anatomy of the meninges, investigators have assumed that the amphibian meninges consist of dura and "secondary meninx" separated by discontinuous subdural spaces. The acceptance which had been accorded Sterzi's conceptions depends mainly upon the fact that although his work is not entirely satisfactory it is, as Dempster ('30) has said, the most complete available. Sterzi's conclusions, however, were based upon the study of only the spinal meninges in both adults and embryos. It is quite evident now that any attempt to homologize the meninges of lower vertebrates with those of mammals must depend upon the modern experimental and embryologic studies of the development of the cranial meninges in man and in the pig which were reported by Weed in 1917.

Following Weed's work, Harvey and Burr ('26) and particularly Flexner ('29) have described the development of the meninges in Ambystoma. These investigators agreed that dura, arachnoid, and pia are all present in amphibians. In both papers the authors described the dura as being closely applied to, if not identical with the internal perichondrium of the skull. Furthermore, Harvey and Burr wrote (p.

551), "Without resorting to experimental methods to determine absolutely the fact, the indications are that the space between the arachnoid and pia is a quite typical subarachnoid space. It is further suggested that the interval between the arachnoid and dura, which is also quite obviously a fluid space, forms a somewhat enlarged subdural cavity." According to this interpretation, which has been followed in the preceding account of the meninges of the toad (fig. 1), the membranes investing the amphibian brain are not essentially different from those of mammals. The dura is a tough fibrous membrane adherent to the inner walls of the skull. A wide subdural space separates it from the delicate, transparent arachnoid, from the internal surface of which fine cellular trabeculae extend across the subarachnoid space to the tenuous pia investing the brain. The homology of these membranes is clear, although the structures are not so elaborately developed as they are in the mammals.

The lining of the subdural space. The literature contains conflicting reports regarding the existence of a mesothelium lining the subdural space. Key and Retzius (1875) demonstrated a mesothelium composed of flattened polygonal cells on the internal surface of the dura in man and the dog. Similarly, Weed ('17), during the course of his studies on the embryology of the meninges in the pig, considered that the presence of a mesothelium on the inner dural surface was the first definite evidence of the appearance of the subdural space. But he noted that silver preparations of the arachnoid failed to reveal a mesothelium on its outer surface. Mallory ('20) asserted that neither dura nor arachnoid has a mesothelial covering, that the dura is lined by flattened fibroblasts, and that the arachnoid is covered by less well differentiated fibroblast-like cells. Kolmer ('25–'26) took issue with this concept and demonstrated typical mesothelium on the inner surface of the dura of the monkey. More recently, Leary and Edwards ('33) reported on the dissimilarities between the lining of the subdural space and that of the serous cavities. They could not demonstrate mesothelium covering the walls of the subdural space and concluded that the dura was lined by naked fibroblasts. Their descriptions, however, are of limited value because they neglected to mention the source of their material and they did not study reduced silver preparations.

Both Sterzi (1899, '00–'01) and Kappers, Huber, and Crosby ('36) describe a layer of mesothelium covering the arachnoid (dura, according to their concept) of amphibians. Neither of these authors discloses the methods by which he derived this information. In my specimens silver preparations failed to reveal a mesothelium lining the subdural space, although preparations of pericardium made simultaneously with the same method were successful. True epithelial cells were also not evident in sections through the meninges. In the toad, therefore, the subdural space resembles a very extensive tissue space bounded by flattened fibroblasts. It contains not only the saccus endolymphaticus, but also a special cell, the meningocyte.

The meningocyte. The cells to which Cushing ('26) applied the term "meningocyte" are probably histiocytes interspersed among the cells lining the subarachnoid space of mammals. According to Cushing's account (p. 33), they lie "here and there in clusters, notably in association with the villi, in nests incorporated within the fibrous mesh of the dura, in the interforaminal prolongations of the arachnoid enveloping the posterior root ganglia, and in the leptomeningeal stalk of the (chorioid) plexus." Functionally and morphologically they resemble the Kupffer cells in the liver and the histiocytes in loose connective tissue elsewhere in the body. They are concerned with phagocytosis of blood cells and debris which may appear in the subarachnoid space, and they produce bile pigments. In response to injected foreign particulate matter they may multiply and round up, and perhaps even become free cells. Thus, these cells constitute the meningeal portion of the reticulo-endothelial system. There is then nothing distinctively meningeal in their character except possibly their location.

The meningocyte of the toad, on the other hand, is distinguished from other cells of amphibian tissues by its peculiar acidophil inclusions. It, too, is evidently a phagocyte, as the nuclear pattern and the ordinarily numerous cytoplasmic inclusions suggest and as the response to injected particulate matter demonstrates. But, it is, at best, a sluggish phagocyte, which is aroused only after prolonged and severe stimulation. In this respect it resembles the histiocyte of connective tissues elsewhere. The presence of fine argyrophil fibers in the cytoplasm of the meningocyte indicates that in addition it is similar to the reticular cells lining the sinusoids of the spleen and the bone marrow.

Contrary to the opinion of Scharrer ('35), the meningocyte of the toad cannot be considered as principally an erythrophage. Its characteristic acidophil inclusions give negative reactions to the histochemical tests which are ordinarily employed to demonstrate erythrocytes or hemoglobin. The exact nature of the meningocyte granules remains in question. It seems most likely, however, that they are protein granules, possibly the residue of earlier phagocytosis, which have been stored and are being slowly digested. The sluggishness with which the cells respond to foreign matter may be due to the fact that they are already full of inclusions. The few mitoses which I have observed have been in meningocytes containing only a few small and poorly staining granules.

SUMMARY

The meninges of the toad consist of pia, arachnoid, and dura, separated from one another by subarachnoid and subdural spaces. The dura adheres closely to the inner walls of the cranium and the vertebral canal. The arachnoid is a thin, transparent membrane connected by cellular trabeculae to the delicate pia which invests the central nervous system.

The subdural space is lined by flattened fibroblasts and no mesothelial layer can be demonstrated.

On the external surface of the arachnoid lie numerous inclusion-bearing cells which have been named meningocytes because they are evidently peculiar to this membrane. These cells are capable of phagocytosing small amounts of foreign particulate matter, such as India ink or blood. Their cytoplasm contains fine argyrophil fibers. The nature of the acidophil inclusions characteristic of the meningocytes remains undetermined, but they can no longer be considered as remnants of ingested erythrocytes, as had been previously thought.

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THE PERSISTENCE OF DECIDUOMATA IN THE MOUSE[1]

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TWO PLATES (THIRTEEN FIGURES)

Since the original experimental production of the deciduomal reaction by Loeb in 1907, this reaction has been considered in many quarters to be at least the morphologic equivalent of the maternal placenta. If this assumption is correct, it may be that an understanding of the factors involved in the production and control of deciduomata will contribute significantly to our knowledge of the processes involved in the establishment and maintenance of pregnancy. As regards production (Loeb, '07, '08) observed that deciduomata could be elicited only during the stages of the sex cycle which are characterized by the presence of corpora lutea. More recently it has been shown that progesterone is the only ovarian hormone necessary for the formation of deciduomata (Rothchild, Meyer and Spielman, '40; Selye, '40; Hooker, '41). It has been shown also that androgen and small amounts of estrogen are not incompatible with the formation of deciduomata in progesterone-treated animals (Brooksby, '38; Rothchild, Meyer and Spielman, '40; Fluhmann and Laqueur, '42).

The hormonal factors concerned in the maintenance and regression of deciduomata are apparently unknown, except for the report of Selye, Borduas and Masson ('42) that deciduomata are not maintained indefinitely in spayed rats receiving daily injections of large amounts of progesterone. Since the animals studied by these authors were examined after complete regression of the deciduomata, the length of the survival period is unknown.

The present investigation was conducted to determine the life span of deciduomata in the mouse and the effects of ovarian hormones upon the survival of this structure.

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[1] The data in this paper were presented as part of a dissertation to the Faculty of the Graduate School of Yale University in candidacy for the degree of Doctor of Philosophy. The author wishes to thank Dr. Charles W. Hooker for his valuable assistance during the course of the investigation.

[2] The manuscript was prepared at the Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York.

MATERIALS AND METHODS

A total of sixty-three female hybrid mice, 60 to 90 days of age, was used in these experiments. One group of twenty-seven of these animals was made pseudopregnant by mating with vasectomized males. On the third day after copulation, as determined by the presence of a vaginal plug, the uteri were traumatized by placing a longitudinal suture in each cornu. Thirteen of these twenty-seven mice received no treatment and were sacrificed at daily intervals through the succeeding 9 days. The remaining fourteen pseudopregnant animals were given daily injections of 1.0 to 2.0 mg. of progesterone for various periods (table 1), and were autopsied 1 to 3 days after the last injection.

TABLE 1

Effect of progesterone (1 mg. daily) upon the survival of deciduomata in intact, pseudopregnant mice.

PERIOD TREATED (AFTER TRAUMATIZATION)	DAY AUTOPSIED (AFTER TRAUMATIZATION)	CONDITION OF DECIDUOMATA
A. Treatment started on day of traumatization and continued until autopsy		
Day 0–9	10	Antimesometrial half necrotic
0–11	12	Wholly necrotic except under myometrium
0–13	14	None present [2]
B. Treatment started after traumatization and continued until autopsy		
3–7	8	Slight necrosis
3–7	8	Slight necrosis
4–8	9	None present [2]
3–4; 5–8 [1]	9	Slight necrosis
3–5; 6–9 [1]	10	Antimesometrial half necrotic
3–13	14	Wholly necrotic except under myometrium
2–11; 12–15 [1]	16	Wholly necrotic except under myometrium
C. Treatment started after traumatization and discontinued		
3–7	9	Much necrosis
3–7	9	None present [2]
3–7	10	Wholly necrotic
3–7	10	Much necrosis

[1] Daily dose increased to 2.0 mg.

[2] Deciduomata may not have formed.

A second group of thirty-six animals was bilaterally ovariectomized. Beginning 2 to 12 days after ovariectomy these animals were given daily injections of 1 mg. of progesterone, and the uteri were traumatized by the insertion of longitudinal sutures on the third day of treatment. From 4 to 16 days subsequent to traumatization, fifteen of these animals were sacrificed. The remaining twenty-one animals in this group were given supplementary daily injections of estrogen or androgen, or estrogen and androgen beginning on the third day after traumatization (table 2).

TABLE 2

Effects of estrogen and androgen on deciduomata in ovariectomized mice receiving progesterone (1.0 mg. daily).

DAILY SUPPLEMENTARY TREATMENT	NO. ANIMALS	DAY AUTOPSIED (AFTER TRAUMATIZATION)	CONDITION OF DECIDUOMATA
A. None	6	4	No necrosis
	2	7	Very slight necrosis
	2	8	Slight necrosis
	1	10	Slight necrosis
	2	12	Almost wholly necrotic
	1	14	Completely necrotic
	1	16	None present
B. 0.5 µg. EB[1]	2	12	Wholly necrotic except under myometrium
	2	14	Absent in one; wholly necrotic in other
	2	16	Wholly necrotic except under myometrium
C. 10.0 µg. TP[2]	2	12	Wholly necrotic except under myometrium
	3	14	Wholly necrotic except under myometrium
D. 0.5 µg. EB + 10.0 µg. TP	3	14	Wholly necrotic except under myometrium
E. 10.0 µg. EB	2	7	Slight necrosis
	2	8	Slight necrosis
F. 250 µg. TP	3	7	Very slight necrosis

[1] EB, Estradiol benzoate.

[2] TP, Testosterone propionate.

The hormone substances were dissolved in sesame oil in such concentration that the daily dose of each was contained in 0.05 cc. or 0.1 cc. of the oil. All of the injections were given subcutaneously. The animals were killed with illuminating gas and examined promptly. The genital tract was fixed in Lavdowsky's fluid. After imbedding in paraffin, sections were cut 8 to 12 micra in thickness and stained with Ehrlich's hematoxylin and triosin or acid eosin.

OBSERVATIONS

Untreated, pseudopregnant animals

Little or no evidence of deciduomal response was visible on the first day after traumatization of the uterus of the untreated, pseudopregnant mice. During the second and third days the tunica propria underwent great local hypertrophy and differentiation into masses of deciduomal tissue (fig. 1). The reaction attained its maximum development on the third and fourth days.

Almost simultaneously with reaching their maximum size the deciduomata underwent retrograde changes. On the third day the sinusoids in the central portion of the deciduoma become engorged and the neighboring tissues were edematous. On the fourth day the engorgement of the sinusoids extended centrifugally toward the myometrium. Islands of deciduomal cells in the central portion of the deciduoma began to manifest cytological characteristics of degeneration, i.e., fragmented nuclear chromatin, vacuolized cytoplasm, and indistinct cell boundaries. A few polymorphonuclear leucocytes and phagocytes were present. The process of regression was extremely rapid, 24 hours later (that is, on the fifth day) the area of degeneration was so extensive that only a few strands of recognizable deciduomal tissue remained under the myometrium. The remainder of the deciduoma consisted of cellular debris invaded by large numbers of lymphocytes, polymorphonuclear leucocytes, and phagocytes (fig. 2). The necrotic mass was gradually circumscribed during subsequent days by a hyperplastic endometrium surrounded by newly regenerated epithelium in such fashion that the mass came to lie in the newly established uterine lumen. This process of encirclement was not complete on the ninth day.

Deciduomata in the untreated, pseudopregnant animals thus survived intact for approximately 4 days from the date of traumatization.

Pseudopregnant animals receiving progesterone

In the pseudopregnant animals receiving injections of progesterone until the day before sacrifice (table 1, A and B) the onset of regressive changes in the deciduomata was delayed until the eighth or ninth day after traumatization (fig. 3) in contrast with the beginning of necrosis on the fourth day in the untreated animals. On the eighth or ninth day in the progesterone-treated animals, the regressive change in the deciduomata was no more than a slight central necrosis. From this date onward, however, despite the continued administration of progesterone, the degeneration progressed rapidly. With the exception of a small area near the mesometrium and adjacent to the metrial gland, the deciduomata were completely necrotic on the twelfth day after traumatization (fig. 4). The mesometrial portion persisted in an apparently healthy condition until at least the sixteenth day. Encirclement of the necrotic deciduomal mass by endometrial epithelium began on the ninth or tenth day. This process was slow, and was still incomplete on the sixteenth day.

On the other hand, when the administration of progesterone was discontinued on the seventh day after traumatization (table 1, C), the process of regression was rapid. On the second day after cessation of treatment the deciduomata were almost completely necrotic and were contained within the re-established uterine lumen.

Ovariectomized animals

In the ovariectomized animals receiving progesterone, the deciduomata were intact and showed no retrograde change on the fourth day after traumatization of the uterus. On the seventh day very slight necrosis was present. On the eighth and the tenth days the extent of necrosis had increased somewhat, but still remained slight (fig. 5). On the twelfth day the deciduomata showed advanced necrotic change (fig. 6). On the fourteenth day the necrosis had involved the whole of the deciduomata, and on the sixteenth day deciduomal tissue was no longer present. The survival period of deciduomata in ovariectomized mice receiving daily injections of progesterone was thus essentially the same as in pseudopregnant mice given injected progesterone. The nature and rate of regression were also similar in the two groups of animals.

To determine whether small supplements of estrogen and androgen would prolong the survival of deciduomata in ovariectomized mice receiving injections of progesterone fourteen animals were employed. Beginning on the third day following traumatization of the uterus, six of these animals were given a daily injection of 0.5 microgram of estradiol benzoate in addition to the daily injection of progesterone (table 2, B). These animals were examined on the twelfth, fourteenth and sixteenth days after trauma. The deciduomata were wholly necrotic except for a small area adjacent to the myometrium (fig. 7). The re-epithelialization of the endometrium was distinctly slower than in the ovariectomized animals receiving only progesterone.

In five animals the daily injection of progesterone was supplemented by the daily administration of 10.0 micrograms of testosterone propionate beginning on the third day after traumatization of the uterus (table 2, C). On the twelfth and fourteenth days the deciduomata of these animals were like those of the animals given supplements of estrogen (fig. 8). They were completely necrotic except for a small area adjacent to the myometrium.

Three animals were given 10.0 micrograms of testosterone propionate plus 0.5 micrograms of estradiol benzoate in addition to the progesterone, beginning also on the third day after traumatization of the uterus (table 2, D). On the fourteenth day after the uterine trauma the deciduomata were like those of the animals receiving but one or neither of these supplementary substances (fig. 9).

Small amounts of estrogen, or androgen, or both simultaneously, therefore, did not prolong the survival of deciduomata in ovariectomized mice receiving progesterone.

To determine whether larger amounts of estrogen or androgen would shorten the survival of deciduomata, seven animals were employed. In addition to the daily injection of progesterone, 3 days after traumatization of the uterus a daily injection of 10.0 micrograms of estradiol benzoate was given to four animals (table 2, E), and of 250 micrograms of testosterone propionate was given to three animals (table 2, F). These animals were examined on the seventh or the eighth day after uterine traumatization. The deciduomata showed no more than barely perceptible necrosis (figs. 10 and 11). Indeed the deciduomata in the animals receiving the supplement of androgen were among the largest observed in the entire study. These amounts of estrogen and androgen apparently did not hasten the regression of the decidual reactions.

DISCUSSION

In untreated mice deciduomata produced by trauma on the third day of pseudopregnancy attained their maximum size in 3 or 4 days, and immediately began to regress. The regression proceeded with such rapidity that they were completely necrotic within 24 hours. The period of growth of the deciduomata in these animals is also a period of apparently increasing level of progestin in uninterfered pseudopregnant mice (Atkinson and Hooker, '43). The necrosis of deciduomal tissue was completed well within the period of pseudopregnancy in this species (usually 10 days). In the latter part of the period of regression, however, the level of progestin has apparently begun to decline somewhat in pseudopregnant mice (Atkinson and Hooker, '43).

The administration of progesterone to pseudopregnant mice beginning at or within 3 days following uterine traumatization prolonged the life of the deciduomata, the onset of regression being delayed until the tenth day following traumatization. This represents at least a doubling of the life of the deciduomata over that in the untreated pseudopregnant animal, and extends the persistence of the deciduoma beyond the span of pseudopregnancy. The process of regression, when it set in, in these animals proceeded almost as rapidly as in untreated pseudopregnant mice and required less than 48 hours.

The persistence of deciduomata in ovariectomized mice receiving progesterone was identical with that in pseudopregnant mice given progesterone. This observation virtually rules out the ovary as being responsible for the failure of complete maintenance of deciduomata in the latter group for more than 10 or 12 days. Supplements of estrogen or androgen in progesterone-treated mice likewise failed to extend or to shorten the persistence of deciduomata.

In the mouse it appears, therefore, that deciduomata have an inherent life-span of 10 days which can be realized only if adequate progesterone is present. This life-span appears to possess a degree of fixity inasmuch as it is not modified by estrogen or androgen.

On the basis that implantation occurs on the fourth day in the pregnant mouse (Snell, '41) and parturition on the twentieth or twenty-first day, the normal life-span of the decidual portion of the placenta would be presumed to be 16 or 17 days. This is a conspicuously longer period of survival than that of deciduomal tissue. A more or less equalizing factor is the fact that the decidual tissue at the original site of the implantation of the ovum is not the definitive decidua basalis. The former is located on the antimesometrial side of the uterus; the definitive decidua basalis forms at least 4 days later (that is, on the eighth day of pregnancy), and lies on the mesometrial side of the uterus. Thus the disparity between the length of survival of the decidua and of the deciduoma is not as great as it appears at first glance.

SUMMARY

Deciduomata produced on the third day of pseudopregnancy in the mouse survived intact for 4 days and then rapidly became necrotic. Injections of progesterone prolonged the survival period of deciduomata in these animals to the point that marked retrogressive changes did not appear before the tenth day after traumatization. Deciduomata in progesterone-treated ovariectomized mice also survived for 10 days. Supplementary administration of estrogen and androgen to ovariectomized mice receiving progesterone did not modify the time of the onset

of regression. These observations suggest that the deciduoma has an inherent life-span which is realized only if adequate progesterone is available.

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PLATES

PLATE 1

EXPLANATION OF FIGURES

Photomicrographs (× 8.2) of deciduomata under various experimental conditions indicating the effects of the ovarian hormones on their persistence. The intact deciduoma (figs. 1, 3, 5, 10, and 11) may be recognized by the organization of the tissue into cords surrounded by sinusoids. The necrotic deciduoma (figs. 2, 4, 6, 7, 8, and 9) appears as a homogenous mass in which little structure can be recognized.

Figures 1 to 4 illustrate deciduomata in pseudopregnant animals with uterus traumatized on the third day after mating.

1 Third day after traumatization. The deciduomal reaction is composed of healthy tissue.

2 Fifth day after traumatization. The deciduoma is completely necrotic.

3 Eighth day after traumatization. Treated with 1 mg. of progesterone daily from third to seventh day. The deciduoma has persisted intact.

4 Twelfth day after traumatization. Treated with 1 mg. of progesterone daily from time of traumatization to eleventh day. The reaction has regressed completely.

Figures 5 to 11 illustrate deciduomata in ovariectomized animals treated with 1 mg. of progesterone daily from third day before traumatization to day before autopsy.

5 Tenth day after traumatization. The deciduoma is composed of healthy tissue.

6 Twelfth day after traumatization. The reaction has almost completely regressed.

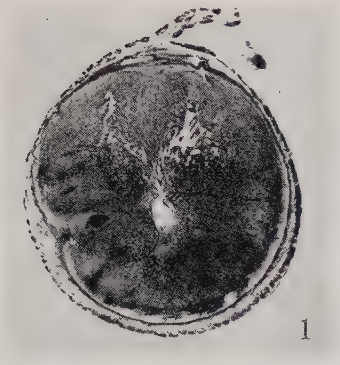
7 Twelfth day after traumatization. Supplementary treatment with 0.5 micrograms of estradiol benzoate daily from third to eleventh day. The reaction has almost completely regressed.

8 Twelfth day after traumatization. Supplementary treatment with 10 micrograms of testosterone propionate daily from third to eleventh day. The reaction has almost completely regressed.

9 Fourteenth day after traumatization. Supplementary treatment with both 0.5 micrograms of estradiol benzoate and 10 micrograms of testosterone propionate daily from third to thirteenth day. The reaction has almost completely regressed.

10 Seventh day after traumatization. Supplementary treatment with 10 micrograms of estradiol benzoate daily from third to sixth day. There is little evidence of regression.

11 Seventh day after traumatization. Supplementary treatment with 250 micrograms of testosterone propionate daily from third to sixth day. There is little evidence of regression.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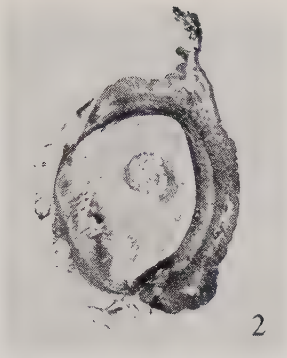
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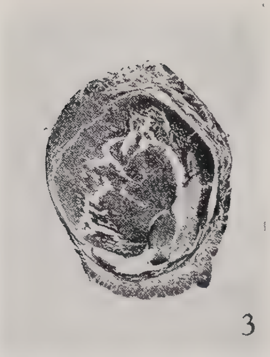
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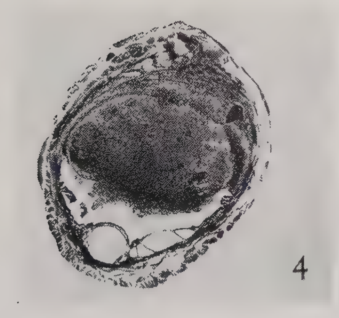
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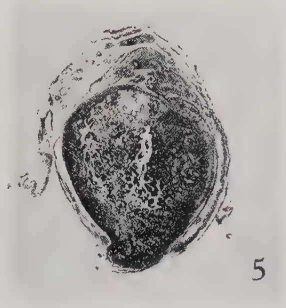
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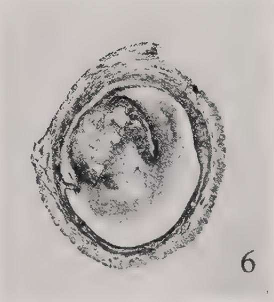
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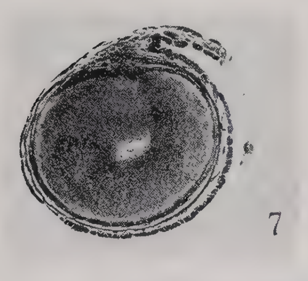
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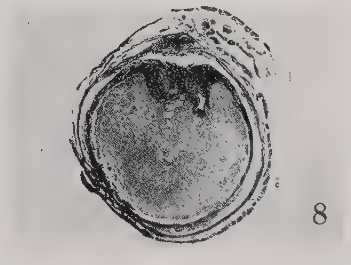
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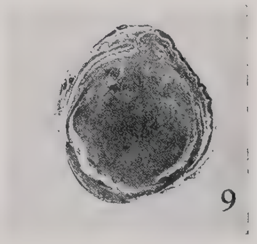
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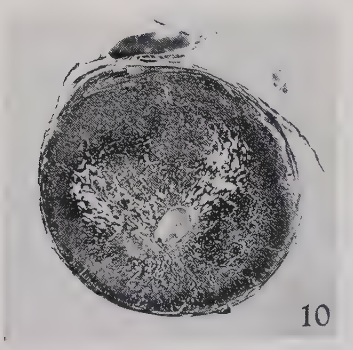
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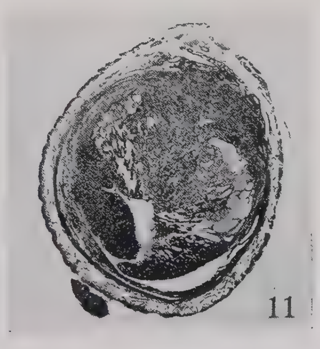
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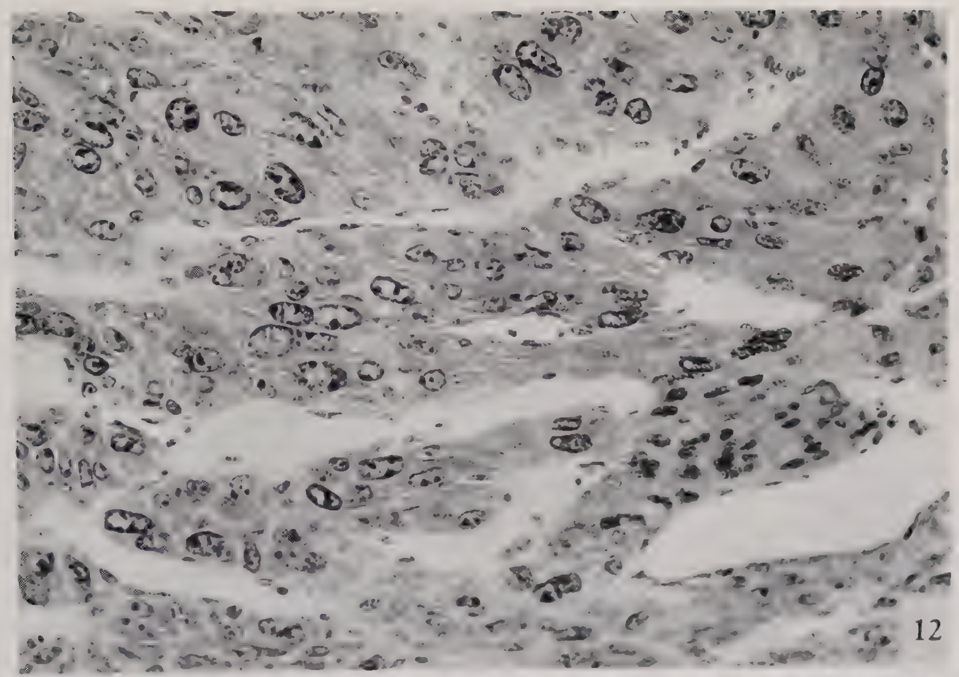
PLATE 2

EXPLANATION OF FIGURES

12 Higher magnification of the central area of the deciduoma shown in figure 1 to illustrate the histological characteristics of healthy deciduomal tissue. Note columns of cells with large vesicular nuclei, surrounded by sinusoids. (× 520).

13 Higher magnification of the central area of the deciduoma shown in figure 7 to illustrate the histological characteristics of deciduomal regression. Deciduomal structure is not recognizable. The cell boundaries are indistinct and the nuclei are pyknotic. (× 520).



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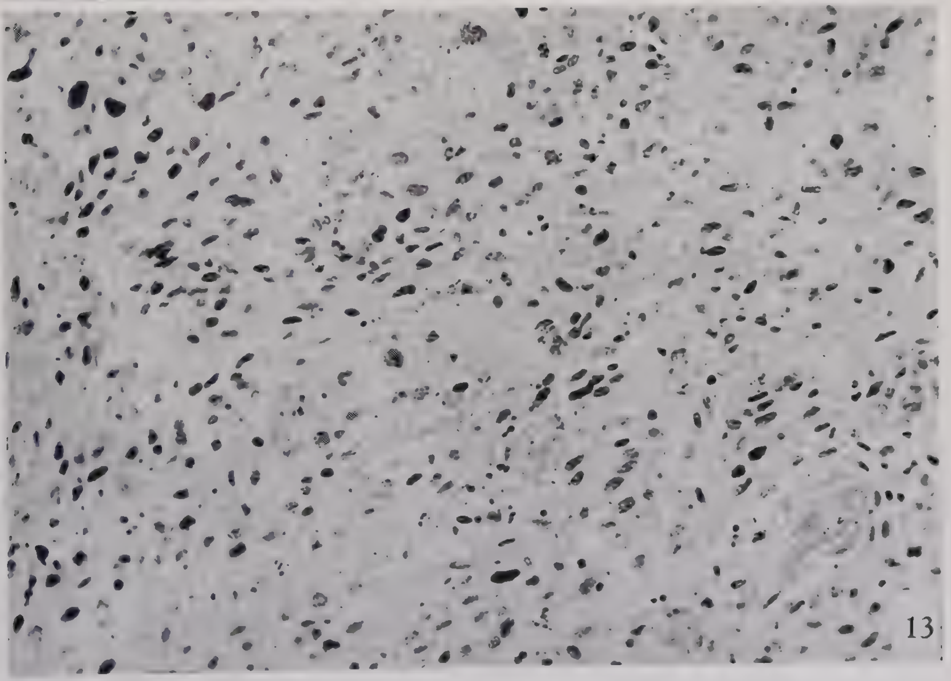

13

BEHAVIOR OF RADIO-IODINE IN RESTING AND STIMULATED THYROIDS

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ONE PLATE (SIX FIGURES)

Sections of thyroids from animals treated with radioactive iodine may affect photographic plates (Hamilton et al., '40; Gorbman and Evans, '41). When such photographic reactions (autographs) were compared under the microscope with the thyroid sections producing them, it was found that radio-iodine is incorporated into the colloid masses of the gland very soon after its injection. Furthermore, differential staining of the thyroid sections with Masson's trichrome stain revealed the presence of a greater amount of the newly-entered radio-iodine in the acidophilic than in the basophilic colloid (Leblond, '43, a, b). This result had to be reconciled with the consensus of opinion linking acidophilic follicles with phases of ''storage'' or relative inactivity of the thyroid; while basophilic follicles are usually considered as indicative of a stimulated gland. In an attempt to understand why more iodine is present in the supposedly less active acidophilic follicles, the amount of radio-iodine taken up by the two types of colloid was examined in the resting thyroid of hypophysectomized animals and in the activated thyroid produced by treatment with pituitary extract.

EXPERIMENTAL

Three groups, of four 150 (±10) gm. male rats each, were used. The first served as control. Animals of the second group were hypophysectomized 2 weeks prior to the injection of radio-iodine. The rats in the third group received two daily injections of 0.25 cc. of the hypophyseal thyrotropic hormone Antuitrin-T for 3 days before the radio-iodine treatment. All animals received a single intraperitoneal injection of 2. micrograms of sodium iodide containing approximately 2. microcuries of radio-iodine (I^{131}, with a half-life of 8 days). The animals were sacrificed 15 hours later. The thyroids were fixed in Susa, embedded, cut at 10 μ, and mounted on a slide. This slide was placed in contact with a photographic plate according to the previously described method (Leblond, '43, b).

At the end of a 4-week exposure, the thyroid slides were stained with Masson's trichrome after being left overnight in saturated picric acid used here as mordant. Light green was used as basic stain. It must be noted that comparable results are obtained with the azan stain or with Mallory's trichrome. By all these methods, the acidophilic follicles stain red and the basophilic, blue or green.[1]

The photographic plates were all developed for the same length of time. Examination with the naked eye showed that all glands affected their respective photographic plates to some extent. The autographs were most intense after pituitary treatment and weakest after hypophysectomy. In preparing the illustrations (figs. 2, 4, 6), negatives were obtained by exposing all the autographs for about the same length of time. But, for printing these negatives, the exposure of those from hypophysectomized animals had to be three times as long as in the case of the controls and four or five times as long as in the case of the pituitary treated animals.

TABLE 1

Effect of hypophysectomy and treatment with thyrotropic hormone on the staining affinity and radio-iodine uptake of the colloid masses in the thyroid.

	ACIDOPHILIC COLLOID		BASOPHILIC COLLOID	
	Relative abundance	Intensity on autograph	Relative abundance	Intensity on autograph
Hypophysectomized	2, 3, 4, 4	0–1	0, 0, 1, 2	0
Normal	1, 3, 3, 4	2	0, 1, 1, 3	1
Antuitrin-T treated	0, 1, 1, 2	4	2, 3, 3, 4	3

The autographs were compared to the stained slides with the help of a binocular microscope (figs. 1–6). As reported previously, no reactions were present in cellular groups, but all colloid masses had reacted on the plate. This was true in all three experimental groups, although a few of the autographs in hypophysectomized animals were too weak to be conclusive. The intensity of the reactions produced by the basophilic and acidophilic colloid on the photographic plate was estimated on the base of a 1–4 scale (table 1). The figure given in the table for each group is valid for every individual in the group, as the reactions were fairly similar in each thyroid of every group. In addition, the relative abundance of basophilic and acidophilic colloid was estimated under the low power of the microscope and rated on a 1–4 scale (table 1). Since individual variations were more pronounced here, the relevant figures for each individual gland were reported. The frequent coexistence of both acidophilic and basophilic colloid in one follicle, especially in normals, did not prevent an estimation of the amount of the two types of colloid.

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[1] The acidophilic colloid stains blue with Mallory's phosphotungstic hematoxlylin stain; it may also stain blue with the routine hematoxylin-eosin, a fact which has been overlooked by some investigators (Hertzler, '41).

The treatment with pituitary extract increased the basophilism of the colloid, and the amount of radio-iodine in both types of colloid; while hypophysectomy increased the acidophilism of the colloid, but considerably decreased the intake of radio-iodine in all follicles (table 1).

In addition, comparison of the autographs produced by the two types of colloid in any given gland, whether resting or stimulated, showed that the acidophilic colloid reacted more intensely than the basophilic (fig. 1–6). Among the acidophilic follicles, those staining most intensely and appearing very dense contained the greatest amount of radio-iodine.

INTERPRETATION

The autographic method confirmed that, soon after injection of radio-iodine, the uptake of labeled iodine is greater in the case of stimulated thyroids and smaller in the case of resting thyroids as compared with control glands (Hertz et al., '38, '40; Leblond and Süe, '40, '41; Hamilton and Soley, '40; Morton et al., '41, '42). Nevertheless, whatever the condition of the thyroid, the radio-iodine was more abundant in the acidophilic than in the basophilic colloid.

The presence of more radio-iodine in acidophilic follicles can be explained in two ways: either more radio-iodine entered these follicles or less came out. The first possibility implied that acidophilic follicles incorporated iodine more actively than the basophilic ones. However, these follicles are characteristic of inactive glands, which are known to fix little iodine. Even when, exceptionally, acidophilic follicles were present in activated glands, their low epithelium and small nuclei made them appear less active than the basophilic follicles in the same gland. Therefore, the assumption that acidophilic follicles are more effective in extracting iodine from the blood than the basophilic ones is not in agreement with known facts.

The alternative explanation of the results is that the acidophilic follicles would retain the fixed iodine longer than the basophilic ones, as in the latter the turnover and excretion of iodine would be more rapid. This explanation is supported by the fact that organic iodine is excreted from the thyroid more actively if the gland is stimulated and presumably basophilic (Morton et al., '41); in contrast, the rate of excretion is much reduced in case of the presumably acidophilic glands of hypophysectomized animals (Morton et al., '42).

This relation of the staining affinity of the follicle with the excretion of iodized products may be interpreted in the light of de Robertis' finding ('41) that a greater amount of proteolytic enzymes is present in the colloid of stimulated, presumably basophilic glands than in normal thyroids. These enzymes would facilitate the hydrolysis of the colloid, preliminary to the diffusion of iodized products out of the follicle (Lerman, '42). Thus, the more rapid elimination of iodine from basophilic follicles would be due to a more rapid hydrolysis of the colloid by the enzymes present there.

In conclusion, it may be noted that, while the entry of iodine into the thyroid appears directly dependent on the size and the activity of the cells (Leblond and Süe, '40, '41), the departure of the iodine from the gland seems closely related to the condition of the colloid. But, although the entry and release of iodine proceed at different rates in individual follicles, it seems that both processes are continuously under way in all follicles, even in hypophysectomized animals.

SUMMARY

Fifteen hours after an injection of 2. γ of radioactive iodine to rats, the colloid in thyroids stimulated with pituitary extract contains more radio-iodine than in the case of the controls; while, after hypophysectomy, the amount of radio-iodine fixed by the colloid is considerably smaller than in the controls.

In spite of the predominance of basophilic follicles in stimulated glands and their rarity in resting glands, these follicles always contained less radio-iodine than the acidophilic ones.

This result is interpreted as showing that the turnover and excretion of iodine take place more rapidly in the basophilic follicles.

We are greatly indebted to Dr. Robley D. Evans, Massachusetts Institute of Technology for the radioactive iodine and to Dr. O. Kamm, Parke Davis and Co., for the Antuitrin-T.

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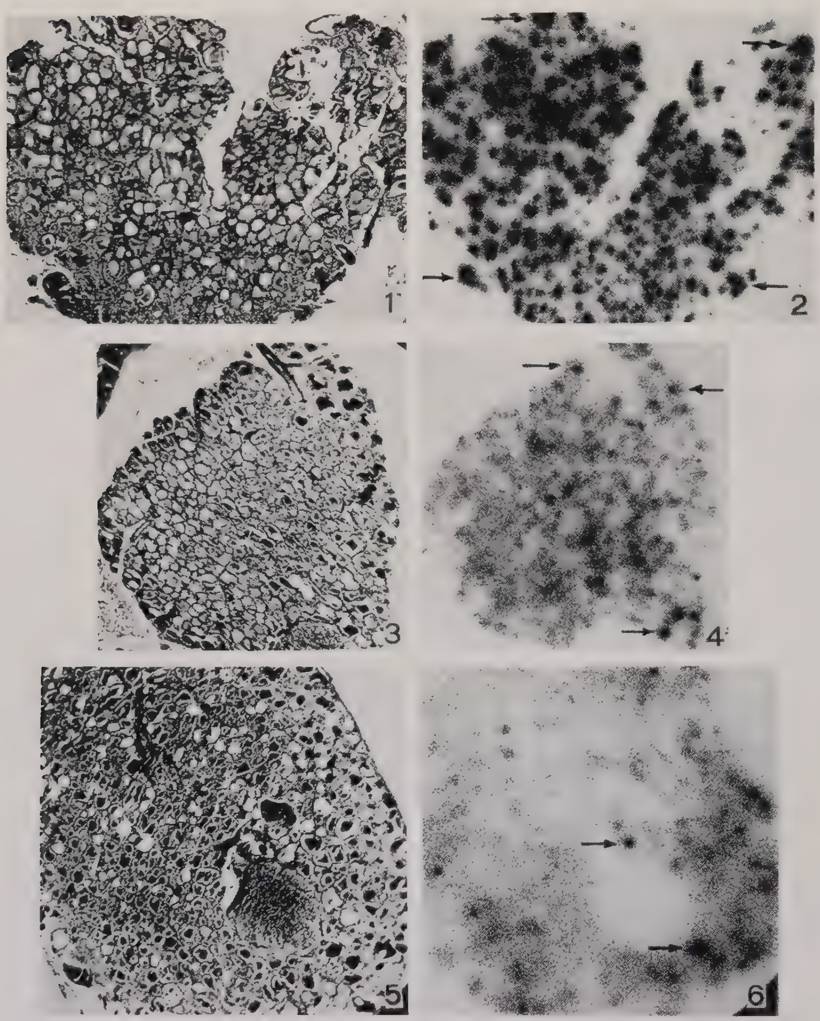

Each microphotograph is accompanied by the corresponding autograph enlarged at the same magnification. Acidophilic follicles appear black on the photograph, basophilic follicles, grey. Vessels filled with red blood corpuscles should not be confused with acidophilic follicles. Arrows point to some of the acidophilic follicles.

1, 2 Thyroid of rat treated with hypophyseal extract.
3, 4 Thyroid of control rat.
5, 6 Thyroid of hypophysectomized rat.

THE HISTOLOGY OF THE MENINGEAL MYELOID TISSUE IN THE GANOIDS AMIA AND LEPISOSTEUS

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and the F. T. Stone Laboratory, Put-In-Bay, Ohio[2]

TWELVE FIGURES

Blood and blood formation in ganoids have been studied by Drzewina ('05; Acipenser), Downey ('09; Polyodon), and Robeson ('32; Amia). Hemopoiesis takes place mainly in the kidneys and the spleen, but also in various other localities. Among these the cranial cavity is of particular interest. There the meningeal connective tissue of the chorioid plexus of the fourth ventricle becomes a hemopoietic organ of considerable size.

Probably the earliest mention of "lymphoid" tissue in the cranial cavity of a ganoid fish occurs in Leydig's textbook of histology (1857; p. 422). Leydig enumerates among lymph glands "the soft, pulpy mass which in the sturgeon covers the anterior part of the spinal cord within the cranial cavity, and which extends up to the roof of the skull."[3] Another early reference to meningeal "lymphoid" tissue within the cranium is found in the paper of Waldschmidt (1887) on the central nervous system of the crossopterygian Polypterus bichir. In this primitive relative of the ganoids the space between the pallium of the forebrain and the cranium is filled with a tissue which Waldschmidt describes as lymphoid and histologically similar to the cephalic kidney of the teleosts.

These authors give no details concerning the cell types which comprise this meningeal "lymphoid" tissue. Drzewina ('05) is the first to describe in the sturgeon granulocytes, lymphocytes, "mononucléaires," and non-granular leucocytes of varying appearance, which together with dark pigment cells are embedded in a reticular stroma.

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[1] This research was aided by a grant made to Western Reserve University by the Rockefeller Foundation.

[2] I wish to thank Dr. Thomas H. Langlois, Director of the Stone Laboratory, and his staff, particularly Dr. Kenneth H. Doan, for their kind cooperation.

[3] "die weiche, pulpöse Masse, welche beim Stör in der Schädelhöhle den Anfangstheil des Rückenmarkes deckt und bis zum Schädeldache emporsteigt."

But still more important, Drzewina already recognizes the tissue as a sort of bone marrow (p. 308): "The lymphoid tissue in the cranial cartilage of the sturgeon appears to be of particular interest, first because its localization is an uncommon one, second because it would seem possible to compare this formation with the bone marrow of the higher vertebrates. For lack of material, I could not deal with the question of the significance of this formation; however, by way of analogy with other better known organs it is probable that this is a kind of cartilage marrow with a hemo-leucopoietic function."[4] Little new was added to Drzewina's description by the papers of more recent investigators; some even contain less or erroneous information.

Chandler ('11) finds the meningeal "lymphoid" tissue only in Lepisosteus, not in Acipenser, Amia, and Polyodon. He concedes that it may have been lost in his specimens of Acipenser and Polyodon in the process of dissection, but he thinks it is lacking in Amia. As it will be seen here in this paper, the tissue mass is present and actually is very large in Amia. Chandler's picture of the histology of the tissue taken from a young Lepisosteus of 25-cm. length is similar to that found in animals which have been kept for some time in captivity without food. In such animals the tissue is depleted of free cells with the exception of granulocytes. From a study of the development of the tissue in embryos Chandler concludes that the "lymphoid" tissue develops from the pia. The paper of Van der Horst ('25) completes the list of ganoids in which this lymphomyeloid tissue has so far been described. He records in Polyodon, Amia, and Acipenser a pigmented tissue mass closely behind the calamus scriptorius, the microscopic structure of which he likens to the spleen; he finds granular and nongranular elements in it, but no erythrocytes. Van der Horst states that he could not find in cyclostomes, selachians, and teleosts a comparable tissue.

Apparently without knowledge of these earlier papers Tilney ('27, '38) describes the same structure in the roof of the fourth ventricle in Amia as a gland of internal secretion. He includes it as the "metaphysis" in his system of glandular outgrowths of the brain (e. g., the epiphysis). Vialli ('32) disagrees with Tilney on this point and emphasizes again the lymphomyeloid character of the meningeal thickening over the fourth ventricle of the ganoids which Drzewina had already stated. Vialli's paper, however, has no figures to illustrate his observations, and it is not clear which role he attributes to this tissue. Jordan ('38) in Downey's handbook of hematology (p. 738) mentions Chandler's paper, but does not ascribe hemopoietic functions to the structure observed by Chandler. Thus there are recorded in the literature various observations which suggest, although only in a general way, a hemopoietic activity in certain regions of the meninges of representatives of all groups of ganoids.

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[4] Le tissu lymphoïde renfermé dans le cartilage céphalique de l'Esturgeon me semble offrir un intérêt tout particulier, d'abord parce que c'est là une localisation peu commune, ensuite, parce qu'il serait possible de rapprocher cette formation de la moelle osseuse des Vertébrés supérieurs. Je n'ai pu aborder, faute de matériel, la question du rôle de la formation en question; toutefois, par analogie avec d'autres organes mieux connus, il est vraisemblable qu'il s'agit là d'une sorte de moelle cartilagineuse, à rôle hémo-leucopoïétique."

The meninges of two ganoid fishes, Amia and Lepisosteus, have been studied, therefore, with this point in mind, and evidence is brought forward to show that blood cells are actually formed in the meninges over the fourth ventricle and are released into the circulation. Judging from the amount of this tissue such a contribution should be substantial and the organ to be described here appears to rank in hemopoietic activity with the spleen and the kidney.

MATERIAL AND METHODS

About sixty specimens of the northern longnose gar (Lepisosteus osseus oxyurus Rafinesque), varying in length from 12 to 58 cm., and of the bowfin (Amia calva Linnaeus) varying in length from 25 to 45 cm. were obtained during the months of May to September from Lake Erie in the neighborhood of the Franz Theodore Stone Laboratory of Ohio State University in Put-In-Bay, Ohio. The fishes were caught in nets (seines, hoop nets, or hand nets), were brought to the laboratory, and were usually fixed there immediately, except in cases indicated in the following pages. It was often possible to fix the animals within ½ hour or less after they had been taken from the net. This is important because there are indications that the conditions of captivity, even if the animals are kept in tanks with running lake water, may influence the histological picture of the blood forming parts of the meninges.

In most cases the fixation was performed by perfusion of the fixing fluid through the vascular system. The fixation of the chromatophores in expansion as illustrated in figure 5a has been taken as a measure of the speedy action of the fixative when perfused through the vascular channels. By contrast the slow penetration of the fixing fluid when the tissue is simply immersed in it seems to give the chromatophores time to contract as is shown in figure 5b.

The large adult specimens can be perfused in the same way as laboratory mammals such as cats or rabbits. The fishes were immobilized by the injection of urethane or nembutal into the abdominal cavity. The heart was exposed and a cannula connected with a percolator taking two liters of fluid was inserted into the heart. The level of the outflow of the percolator was kept at 130 cm. above the heart. The pressure resulting from this height of the perfusion bottle might seem high. But Greene ('04) reports that in eleven male Chinook salmon varying from 42.8 to 93.5 cm. in length the average aortic pressure was 74.3 mm. of mercury which is the equivalent of 101 cm. of water. A number of male and female specimens were found to have a blood pressure above 100 mm. of mercury with a maximum of 120 mm. of mercury or 163 cm. of water. Greene emphasizes that loss of blood and the general conditions of the experiment tend to result in too low figures rather than too high ones. Also in the sections of perfused ganoid material nothing has been observed that would indicate that the pressure used in perfusing the animals has been unsuitably high. No ruptured blood vessels have been seen. The sinusoids, the walls of which are very delicate are of about the same diameter in perfused material as in tissue that had been fixed by immersion (fig. 5a and b). The cells around the sinusoids in perfused tissue do not look as if they had been under excessive pressure, and rounded off macrophages are often found attached to the sinusoid wall (fig. 4), indicating that the fixing fluid did not pass through the vessels with great force. No saline was used and the blood was washed out with plain Zenker's fluid which was followed by Zenker-formol. The fixation fluid was kept flowing intermittently for at least 10 minutes. Then the brain with the meninges and whichever other organs were wanted, were removed and were left in Zenker-formol for another 3 to 6 hours. These tissues were washed in running water for 24 hours.

The material was embedded either in nitrocellulose or over alcohol, methylbenzoate, and benzene in paraffin containing some bayberry wax. The sections were stained with Masson's trichrome stain, Maximow's hematoxylin-eosin-azure, Heidenhain's azan, hematoxylin-eosin, or only with azocarmin. Specimens perfused with alcohol-trichloracetic acid-mercuric chloride [5] were impregnated with Bodian's protargol method. Foot's method for argyrophil fibers was applied to such or Zenker fixed material.

OBSERVATIONS

In both Amia and Lepisosteus the meninges over the fourth ventricle are greatly thickened, forming a large mass of tissue the shape of which is different in the two species studied (fig. 1a, b). Most of the authors who have described brains of ganoid fishes do not mention it; presumably it was lost when the brains were removed from the skull. Those who mention the peculiar outgrowth realized that it is not part of the brain proper. Thus C. L. Herrick (1891) illustrates the brain of the gar pike (Lepisosteus) and writes in the description of the figure: "The bi-lobed mass lying behind the cerebellum is not of a nervous character." As it develops, it apparently fills the space over the fourth ventricle in the same way as the saccus endolymphaticus fills the corresponding space in the amphibia. A comparison of the brain of Lepisosteus with that of Amblystoma (Dempster, '30, fig. 13, p. 123) shows the similar formations resulting in both cases. Neither one of these structures could become permanent in the ascending vertebrate

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[5] I wish to thank Dr. D. Bodian for suggesting this fixing fluid the composition of which is as follows: alcohol 95%, 100 cc.; trichloracetic acid, 1.5 gm.; mercuric chloride, 3 gm.

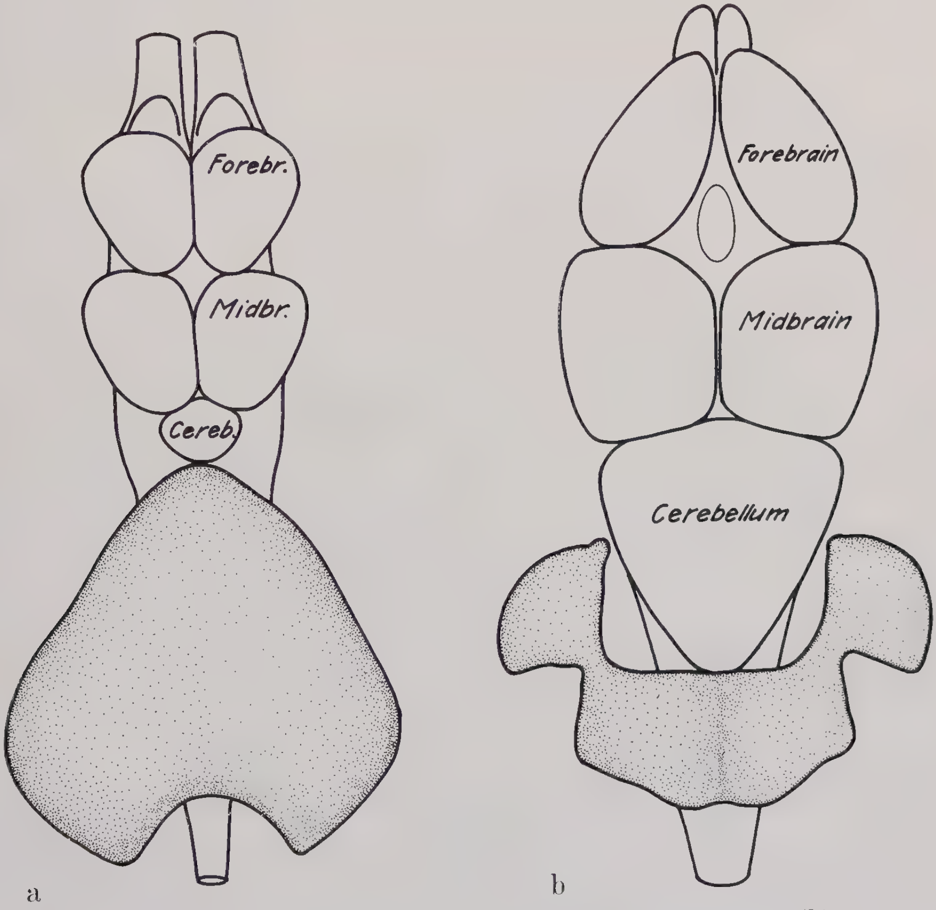

Fig. 1 Myeloid organ (stippled) of Amia (a) and Lepisosteus (b).

series as the space is claimed in the higher vertebrates by the expanding brain.

The histological structure of this organlike tissue-mass (fig. 2a, b), which evidently occurs only in ganoids, has been described but briefly and in general terms by some of the authors cited above. Large chromatophores and granulated cells were seen by the few observers who studied sections, but no adequate illustrations were given.

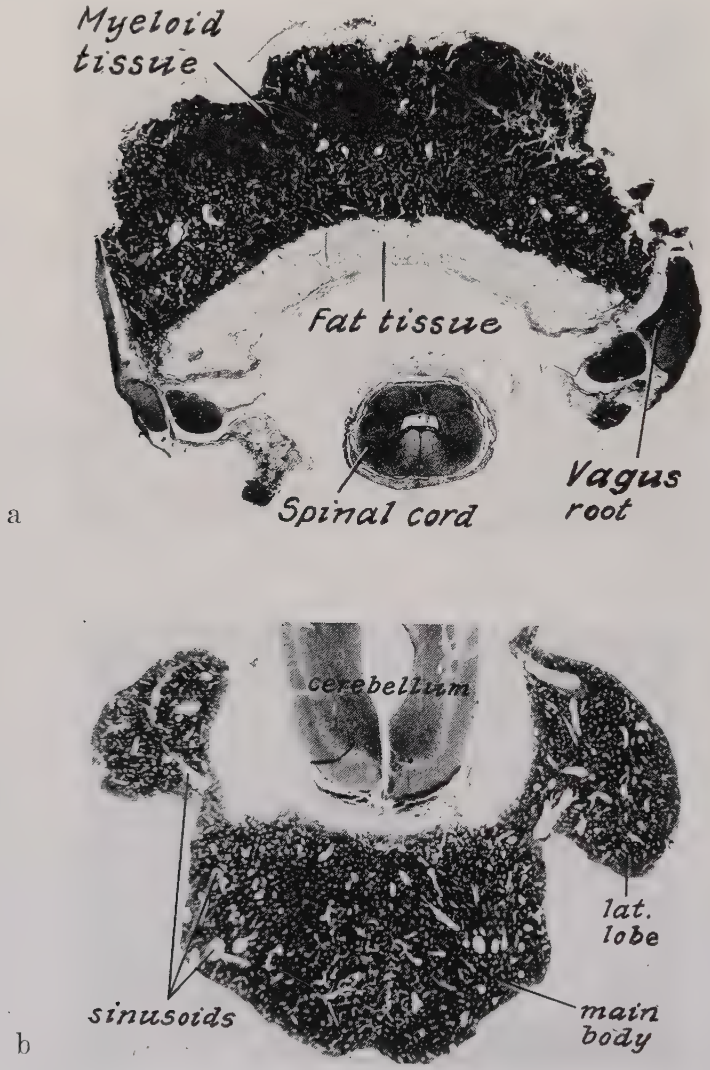
Myeloid tissue
Fat tissue
Spinal cord
Vagus root
a
cerebellum
sinusoids
lat. lobe
main body
b

Fig. 2 a. Cross section through the myeloid organ of Amia. × 7. b. Horizontal section through the myeloid organ of Lepisosteus. × 8. Photomicrographs.

A study of sections prepared with various techniques shows that the tissue under consideration looks like bone marrow consisting of a stroma and free cells. It will be seen in the course of this study that the tissue actually is bone marrow, which here in the ganoids makes its first appearance in the vertebrate series. The following description emphasizes, therefore, points of similarity or difference between the meningeal myeloid tissue of the ganoids and the bone marrow of the higher vertebrates.

Stroma. In sections prepared with the silver technique of Foot (fig. 3) or the azan method of Heidenhain (fig. 4) a fibrous network can be demonstrated the fibers of which are arranged in irregular meshes as in mammalian bone marrow. No significant difference can be detected between the two species studied, as in both the reticular connective tissue is continuous with the perivascular connective tissue, and the amount of fibers and their variation in thickness, etc., appear to be quite similar.

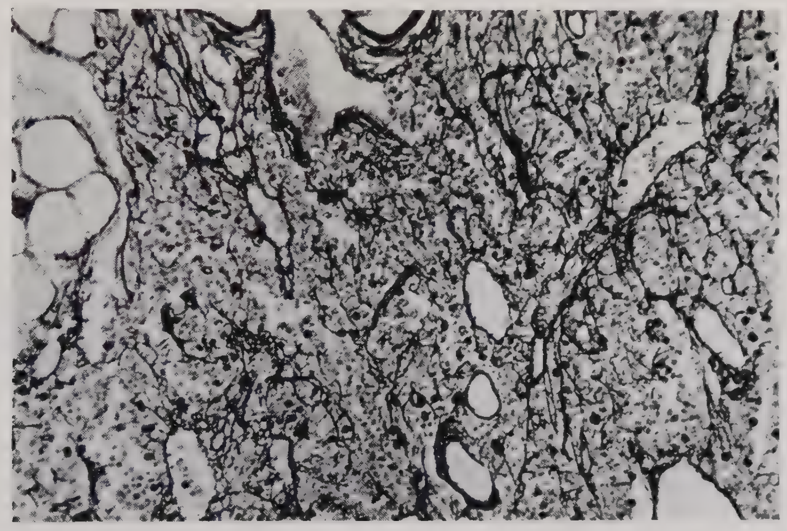

Fig. 3 Reticular fibers in the myeloid tissue of Amia. Fixation by perfusion with alcohol-mercuric chloride-trichloracetic acid (see page 304), paraffin, 8 μ, Foot's silver method, photomicrograph, × 300.

The nuclei of the reticular cells that are attached to the fibers have fine chromatin granules and stain in azan preparations the same blue as the connective tissue fibers in contrast to the red staining nuclei of all the other cells (fig. 4). They share this blue staining in the azan preparation with the nuclei of the littoral cells which have the same chromatin structure as those of the reticular cells. Whereas the littoral cells store trypan blue and phagocytose cellular debris, the reticular cells did not store trypan blue in animals which had received repeated injections of large doses of the dye. This failure of the reticular cells to store trypan blue indicates, according to Maximows' ('32) observations in mammals, that the reticular cells are undifferentiated mesenchymal elements in contrast to the littoral cells, which are fully developed histiocytes. In the ganoids the same interpretation does not necessarily apply; other factors may play a role in preventing the reticular cells from storing trypan blue.

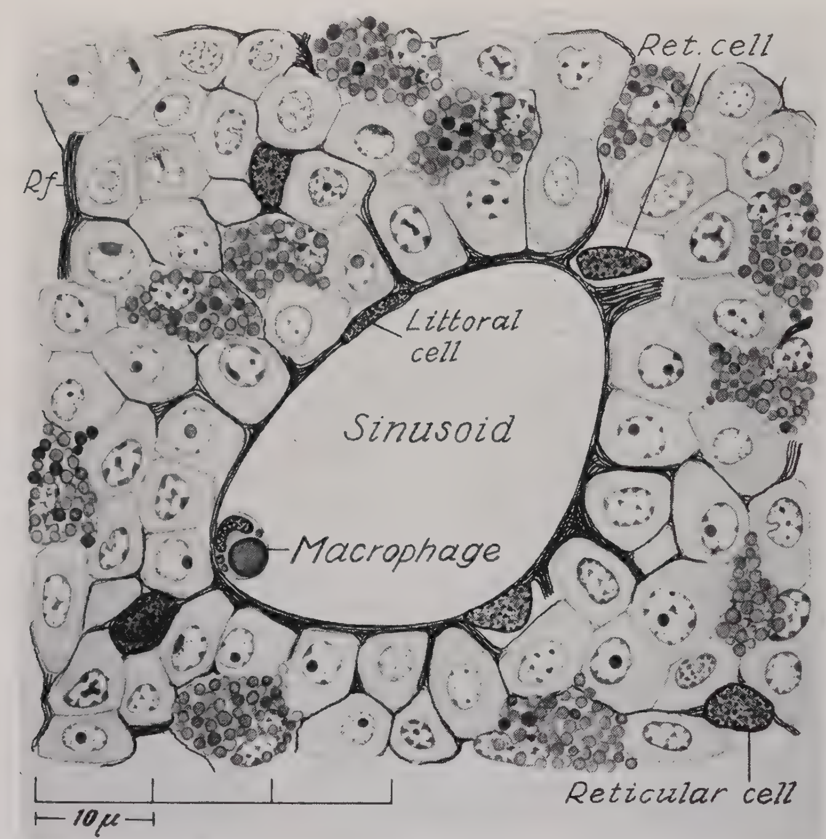

Fig. 4 Myeloid tissue of Lepisosteus. Combined drawing from different parts of a section stained with Heidenhain's azan. The nuclei of the reticular cells, the littoral cells, and the macrophages stain the same blue as the reticular fibers (Rf). The nuclei of all other cells stain orange red. Fixed by perfusion with Zenker-formol, nitrocellulose, 8 μ.

Closely associated with the connective tissue are large chromatophores that contain a dark brown pigment. When expanded their processes follow the fibers of the stroma as can be seen in azan preparations. Their presence in the myeloid tissue is explained by the origin of this tissue from the meninges which, particularly on the dorsal surface of the brain, contain numerous chromatophores of the same kind as those described here. It has been mentioned already (p. 293) that, as a rule, in preparations which were obtained from material fixed by perfusion the chromatophores are expanded with their processes reaching far between the free cells (fig. 5a); whereas in tissue fixed by immersion the chromatophores are contracted (fig. 5b). This observation indicates that during the slow penetration of the fixing fluid the chromatophores in immersed material had time to contract. By contrast, the rapid fixation achieved by perfusion does not permit the contraction of the chromatophores which consequently are fixed in the expanded state. This effect of the different techniques of fixation must be kept in mind when the reactions of these chromatophores and the factors which cause them to expand and to contract are studied.

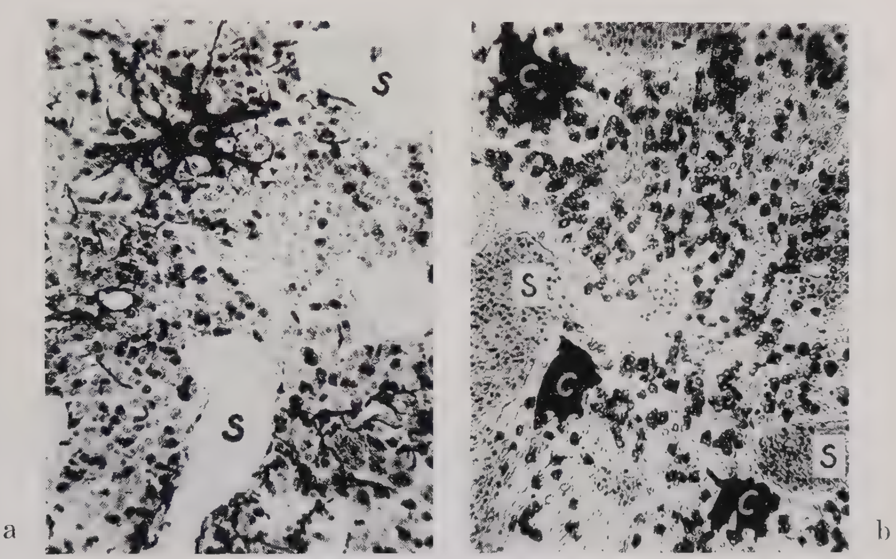

Fig. 5 Sections through the myeloid tissue of Lepisosteus, stained with azocarmine. a. Fixation by perfusion with Zenker-formol. One chromatophore (c) is expanded; the processes of another are visible in the lower right corner. The sinusoids (s) are empty. b. Fixation by immersion in Zenker-formol. Three chromatophores (c) are fully contracted. The sinusoids (s) are filled with blood; their diameter is about the same as in a (see text, p. 294). The numerous dark staining cells in both photomicrographs are granulocytes the granules of which stain with azocarmine. Paraffin, 8μ, × 200.

The blood vessels with which the stroma is intimately connected are arteries, veins, and sinusoids. The arteries and veins are not different from those seen in other ganoid tissues or in tissues of other animals for that matter. The venous sinuses (sinusoids) are as characteristic in the meningeal myeloid tissue of the ganoids as they are in the bone marrow of the higher vertebrates. The walls of the sinusoids are lined by histiocytes (littoral cells; fig. 4). They store trypan blue and are frequently seen rounded off, containing inclusion bodies of various sizes. Evidently they become free macrophages in much the same manner as the histiocytes in the sinusoids of the mammalian bone marrow.

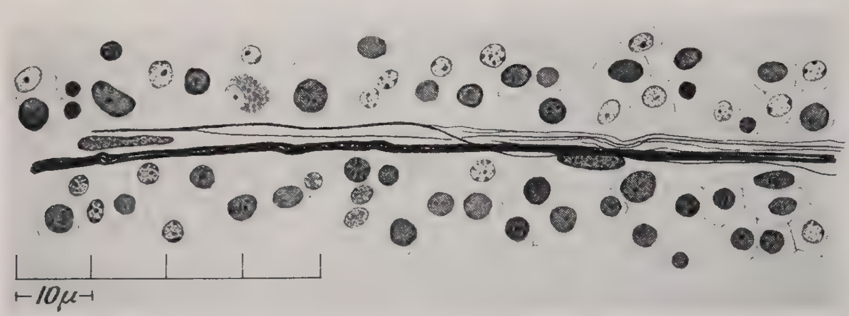

Fig. 6 Nerve fibers with two Schwann cells in the myeloid tissue of Amia. Fixation by perfusion with alcohol-mercuric chloride-trichloracetic acid, paraffin, 8μ, Bodian's protargol method.

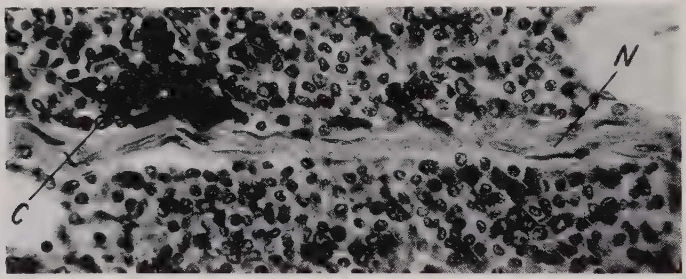

Fig. 7 Nerve fibers (N) with numerous Schwann cells in the myeloid tissue of Lepisosteus. C. Chromatophore. Fixation by perfusion with Zenker-formol, nitrocellulose, 6 μ, hematoxylin-eosin-azure, × 400.

In the bone marrow of the mammals fat cells are said to be always present, scattered singly in the red marrow. In the ganoids, no fat cells are found in the meningeal organ except in a rather narrow marginal zone where fat tissue is interspersed with myeloid tissue. This fat is everywhere in the extensive space between the cartilage of the skull and the brain. It is not comparable to the yellow fatty bone marrow of the higher vertebrates, and there is no indication that the meningeal myeloid tissue becomes fatty in older animals or in specimens that have lived under unfavorable conditions (captivity).

Part of the stroma is formed by nerve fibers. They occur in large and small bundles (figs. 6 and 7) which, as a rule, do not accompany the blood vessels but take an independent course. Single fibers arising from such bundles can be traced between the cells, but no endings of any specific kind have been identified. The pictures given here are identical with the illustrations of the nerve fibers in the bone marrow of birds and mammals in the papers of Rossi ('32) and Takeyama ('36). Whereas nerve fibers are found in both Amia and Lepisosteus, large nerve cells with vesicular nuclei and finely dispersed Nissl bodies were observed in Amia only (fig. 8). These cells are usually encountered single, rarely in pairs, and are scattered widely over the whole extent of the section through the organ.

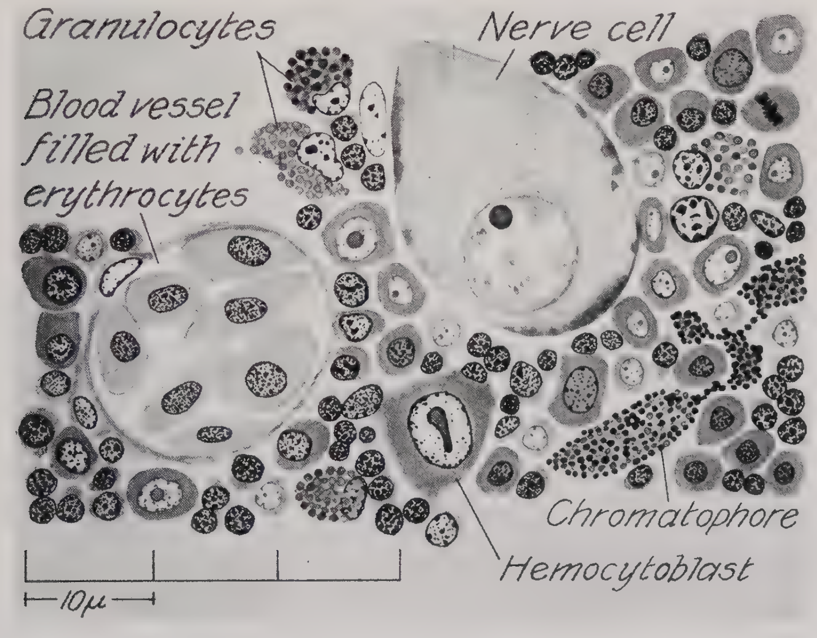

Fig. 8 Myeloid tissue of Amia. Combined drawing from different areas of a section. In addition to the cell types labeled, there are numerous lymphocytes and other agranular free cells which cannot be identified in terms of the mammalian bone marrow. Fixed by perfusion with Zenker-formol, nitrocellulose, 8 μ, hematoxylin-eosin-azure.

Free cells. The spaces within the stroma are filled by free cells. They are briefly characterized here as constituents of the tissue in order to complete its histological description. The tracing of the origin, development, and fate of the various types of free cells has not been attempted in any detail. The results of investigations concerning the life history of erythrocytes, granulocytes, etc., produced in the meningeal myeloid organ, and the role of age and seasonal factors such as temperature, light, food will be reported in subsequent papers. The free cells may be put into two categories, cells with distinct and brightly staining granules, and cells with no or not sufficiently discernible granules.

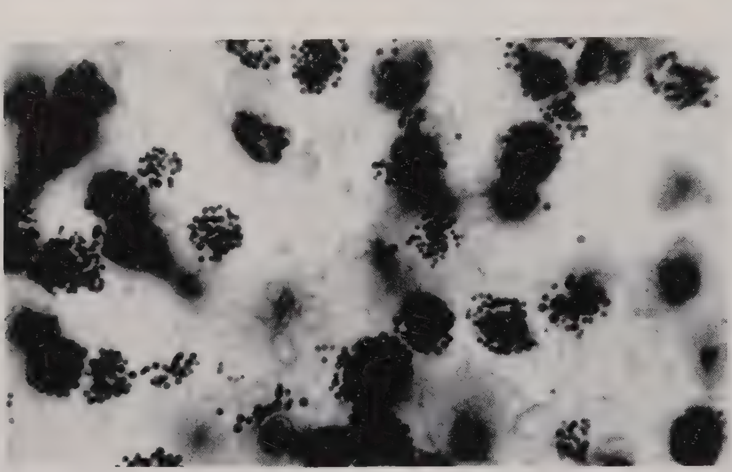

Fig. 9 Section of the myeloid tissue of Lepisosteus showing the affinity of the granulocytes for azocarmine. Fixation by perfusion with Zenker-formol, paraffin, 8 μ, azocarmine, photomicrograph, × 1200.

Turning first to the granulated cells, there are numerous conspicuous granulocytes (fig. 9) which dominate the histological picture and which have been noted also by previous observers. In Lepisosteus they are of oblong shape with a short diameter of about 5 micra and a long diameter of 10 micra in celloidin sections of material perfused with Zenker-formol. In Amia the same cells are smaller with a short diameter of about 3 micra and a long diameter of roughly 5 micra. The granules fill the whole cell body. They stain with eosin in hematoxylin-eosin-azure preparations, although the staining is by far not so bright as that of typical eosinophils of the mammalian blood. More characteristic for these cells seems their great affinity for azocarmin. They also stain brilliantly with the red component of Masson's trichrome stain which contains acid fuchsin and ponceau de xylidine. These granulocytes which will be referred to in the following as eosinophils, were found, although in small numbers, in blood smears. But, because they were not seen in all smears made from different specimens, their actual percentage in the blood was not determined. The cells are not infrequently seen within the sinusoids of the myeloid tissue, but in very few instances have they been observed passing through the walls of the sinusoids. Like the eosinophils of the higher vertebrates these cells are very common in the connective tissue anywhere in the body and have been found in sections through the various abdominal organs such as the intestines.

There is a very small percentage of cells the granules of which are small, do not fill the cytoplasm, and stain purple in eosin-azure preparations. They might correspond to the basophils of the higher vertebrates. These cells have so far been seen only within the myeloid tissue and not in any phase of passing through the sinusoid walls.

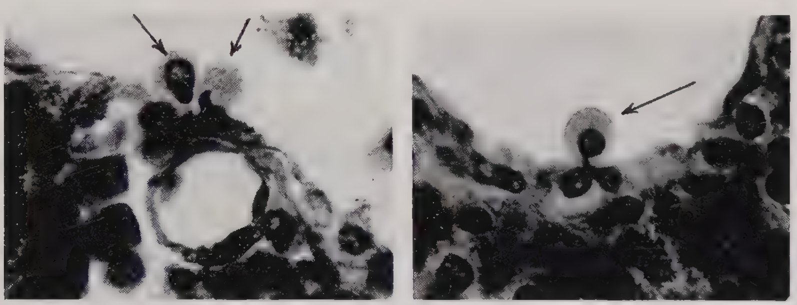

Fig. 10 Agranular free cells on their way into the sinusoids in Lepisosteus. Fixation by perfusion with Zenker-formol, paraffin, 8 μ, hematoxylin-eosin, photomicrograph, × 1200.

Of the cells without granules one type is very common, particularly in Lepisosteus. Its cytoplasm stains pale pink in eosin-azure preparations. If there are any granules in this cell they cannot be demonstrated satisfactorily by the methods used here. Their average diameter in celloidin sections is 5 micra in both Amia and Lepisosteus. This cell is frequently seen passing through the walls of the sinusoids (fig. 10) and may lie free within the blood vessels. It seems that this is a stem cell from which other more differentiated types are derived. The cells are frequently seen in mitosis, and in young gar pikes some of them are observed with only a few granules of the type of the eosinophil cells. Various stages were seen from cells containing a few granules to the mature types filled with eosinophil granules. Apparently this non-granular cell can thus become an eosinophil. Furthermore in some gar pikes from which a considerable amount of blood had been withdrawn (8 to 10 cc.) by cardiac puncture and which were killed at various intervals after bleeding the cytoplasm is basophil in a large number of these cells. It remains to be seen whether this is equivalent to a first step in the development of hemocytoblasts.

Cells having the characteristics of hemocytoblasts occur not only in animals that have lost blood, but are regularly found in normal healthy specimens, although not in great numbers (fig. 8). The nuclei of these cells are large with coarse chromatin particles and big nucleoli. The cytoplasm stains blue in eosin-azure preparations. The cells are sometimes seen in mitosis and have also been observed within the sinusoids.

Erythrocytes are found, single or in clusters, irregularly scattered over the myeloid tissue. In some specimens of Lepisosteus the erythrocytes are encountered in various phases of their passage through the sinusoid walls. As can be seen in figure 11, the nucleus or the cytoplasmic cell body may be within the lumen of the sinusoid ahead of the rest of the cell. For the demonstration of these phases, perfusion with

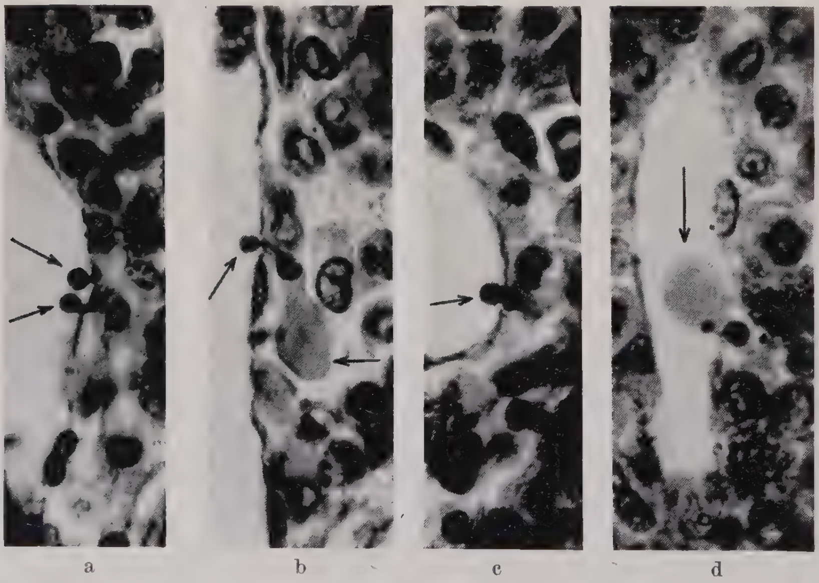

Fig. 11 Erythrocytes on their way into the sinusoids of the myeloid tissue of Lepisosteus. a. Two nuclei (arrows) are just passing through the sinusoid wall. The cell bodies lie in a different plane and are not visible in the photomicrograph. b. Only the smaller part of the nucleus (arrow) is inside the lumen of the sinusoid. It is connected through a tenuous bridge with the larger segment which is still on the other side of the wall of the sinusoid. The cytoplasmic body (arrow) drags behind the nucleus. c. Nucleus of an erythrocyte subdivided in about equal portions by the constriction due to the passage through a very narrow opening in the sinusoid wall. d. Cytoplasmic body (arrow) of an erythrocyte going ahead with a small segment of the nucleus. The larger portion of the nucleus still lies outside the lumen of the sinusoid. Fixation by perfusion with Zenker-formol, paraffin, 8 μ, hematoxylin-eosin, photomicrograph, × 1200.

the fixing fluid is evidently essential. First, perfusion removes all the other cells from the lumen of the vessel so that the part of the erythrocyte that protrudes into the lumen of the sinusoid is clearly discernible. Second, the cell is fixed instantaneously while still in the process of diapedesis. However, if the tissue were fixed by immersion, the cell might have enough time either to slip through entirely or to retract the protruding part before the slowly penetrating fixing fluid reaches the cell.

In the bone marrow of mammals essentially the same process is observed although there are some differences in details. According to Maximow ('10) the ''endothelium'' of the blood vessels in embryonic bone marrow becomes discontinuous in some places permitting the extravasation of blood plasma which flushes the erythrocytes into the circulation. Drinker, Drinker, and Lund ('22) conclude from their observations that the irregularly placed red cells grow through the extremely delicate walls of the sinusoids. Other investigators have proposed still different modes of entry of red cells into the circulation, but they all agree on the extravascular origin of red cells in the adult bone marrow. Thus also in this respect the meningeal myeloid tissue of ganoids is similar to the marrow of higher vertebrates. As a material for the observation of the actual release of mature erythrocytes into the circulation the myeloid tissue of the ganoids has great advantages.

The migration of red cells through the walls of the sinusoids does not occur in all specimens with equal frequency. There are indications which will have to be substantiated by future investigations that erythropoiesis and release of red cells into the circulation depend upon seasonal factors, and that as in the bone marrow of the frog the activity of the myeloid tissue is at its peak in spring.

Greatly varying in their number are small cells which correspond in every way to the lymphocytes of higher vertebrates (fig. 8). The diameter of their deeply staining nuclei is two to three micra in celloidin sections of material from Amia and Lepisosteus. These lymphocytes have been seen entering the sinusoids. In this respect they resemble the lymphocytes which in the embryonic bone marrow of mammals may be washed into the circulation in the same manner as the erythrocytes (Maximow, '10).

As a rule lymphocytes are much more common in Amia than in Lepisosteus. In Amia large areas appear to contain nothing but lymphocytes, whereas in Lepisosteus they are usually single, only about two to five occurring within one field in the microscope. Occasionally they accumulate in greater numbers around a bundle of nerve fibers.

Some of the sinusoids in Lepisosteus are filled with cells which fit the description of the thrombocytes found in amphibia and fishes. The nuclei of these cells are elongated, measuring about 5 micra in length and about 2 micra in diameter. These "thrombocytes" fill symmetrically located sinusoids. Apparently they do not enter the lumen individually as do other cells, but seem to be thrust into the sinusoid en masse. The details of this process have not yet been fully studied.

No detailed description of the free cells has been given in this paper and no attempt will be made to trace their origin and follow their fate as blood cells before additional information becomes available from experimental work. But it is quite obvious that the tissue mass under consideration here is, contrary to Tilney's interpretation, not of glandular but of myeloid character. It becomes bone marrow in the strict sense of the word where it extends into little bony cavities inside the cartilaginous skull (fig. 12a, b). The meningeal myeloid tissue of the ganoids represents the first bone marrow in the vertebrates.

DISCUSSION

As a variety of connective tissue the meninges of the vertebrates are potentially hemopoietic. In fact, Kolmer ('21) mentions that in newborn dogs during the first weeks of life lymphatic elements abound in the connective tissue of the chorioid plexus of the lateral and fourth ventricles. Even megacaryocytes appear and Kolmer compares such areas to small foci of bone marrow. However, only in the ganoid fishes do permanent large lympho-myeloid organs develop in the connective tissue over the chorioid plexus of the fourth ventricle. This meningeal lympho-myeloid tissue becomes bone marrow in a strict sense where it fills bone-lined cavities of the skull. Thus myeloid bone marrow which was supposed not to occur in the vertebrate series below amphibians, actually makes its first appearance in the ganoid fishes.

This myeloid tissue of the ganoids has general histological interest also in other respects. For one, the passage of red cells through the sinusoid wall can be observed. It is to be remembered that the difficulty of explaining the entry of red cells into the circulation was deemed so formidable by Sabin and her collaborators that they considered the intravascular origin of the red cells as the only alternative. "Since the power of locomotion has not been observed in the red cells at any stage in their development, an intravascular origin of these cells gives the only feasible mechanism so far suggested to explain their entrance

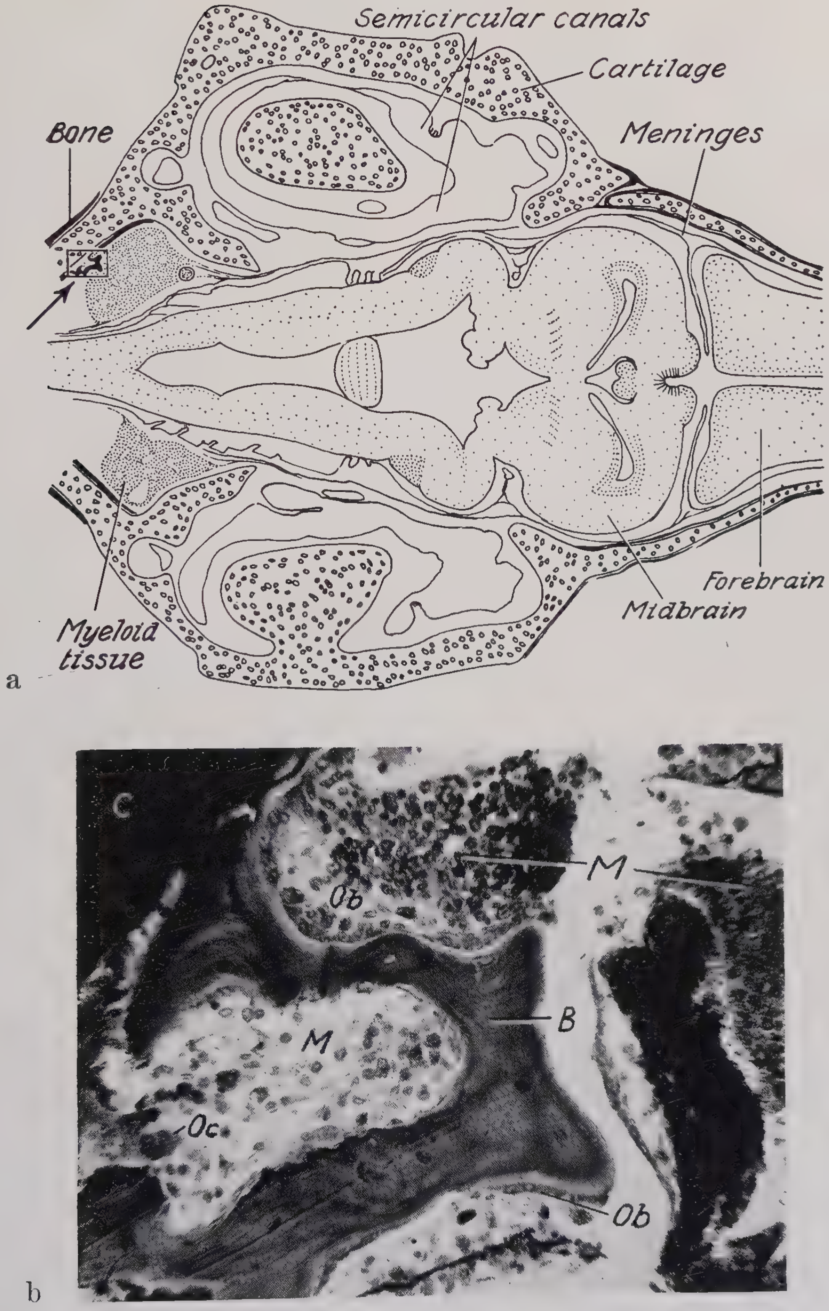
Semicircular canals
Cartilage
Bone
Meninges
Myeloid tissue
Forebrain
Midbrain
a
C
M
Ob
M
B
Oc
Ob
b

Fig. 12 a. Drawing of a horizontal section through the skull and brain of Lepisosteus, × 10, b. Photomicrograph of the area indicated by arrow in the drawing. B Bone, C Cartilage of the skull, M Myeloid tissue, Ob Osteoblasts, Oc Osteoclast. Fixation by perfusion with Zenker-formol, nitrocellulose, 8 μ, hematoxylin-eosin-azure, × 400.

to the vessels of a closed system'' (Sabin, '32, p. 510). The concept of the intravascular origin of red cells in the adult vertebrate bone marrow has not been accepted by the majority of investigators. The observation of red cells passing through the walls of the sinusoids in the myeloid tissue of the ganoids adds much to the weight of evidence in favor of the extravascular origin of erythrocytes in adult myeloid tissue in general.

The question still remains, of course, how red cells move or are moved from the site of origin in the parenchyma to and through the walls of the sinusoids. Nothing noteworthy can be contributed to that problem from the study of the myeloid tissue of the ganoids except that in penetrating the wall of the sinusoid either the nucleus or the cytoplasm of the erythrocyte may go ahead.

Another interesting point concerns the innervation of the meningeal myeloid tissue. The observation of numerous nerve fibers and even of nerve cells within the myeloid tissue of the ganoids stands in marked contrast to the description of the bone marrow of higher vertebrates in text- and handbooks. In Moellendorff's handbook of microscopical anatomy ('27) Maximow says nothing about nerves in his description of the bone marrow. In Downey's handbook of hematology ('38) Doan states (p. 1852) that in the bone marrow nerves cannot be demonstrated satisfactorily, ''and the nervous mechanism of bone marrow stimulation and control has therefore, been thought to be minimal, while chief importance has been attributed to physico-chemical stimuli.'' No mention is made of the innervation of the bone marrow in Maximow-Bloom's textbook of histology ('42).

But Foà ('35) who made an extensive study of the functional innervation of the bone marrow, cites Duverney (1700), Bichat, Gros, Variot and Rémy of the 19th century, and Ottolenghi ('01) and De Castro ('25) as having demonstrated the presence of nerves in bone marrow. Rossi ('32) describes in birds and mammals bundles of nerve fibers which are distributed among the cells of the myeloid tissue. In the same way Takeyama ('36) clearly illustrates with numerous pictures the innervation of bone marrow in birds, mammals, and in man.

The myeloid tissue of the ganoids therefore does not differ from the bone marrow of the higher vertebrates with respect to innervation; both tissues are well innervated. This does not mean that myeloid tissues are under nervous control only; as in other organs probably both nervous and physico-chemical factors govern the activity of the tissue.

SUMMARY

1. The large mass of tissue over the fourth ventricle in the ganoid fishes Amia and Lepisosteus is of myeloid, not of glandular character.
2. Erythrocytes, granulocytes, and lymphocytes are formed in this tissue and are released into the circulation.
3. The passage of mature red cells through the walls of the sinusoids has been observed; erythropoiesis is extravascular.
4. This tissue corresponds not only histologically to myeloid tissue, but also fills small bone-lined cavities of the skull. The ganoids are, therefore, the first vertebrates to have true bone marrow.
5. This myeloid tissue contains numerous nerve fibers and even nerve cells (in Amia). The question of the innervation of the bone marrow of higher vertebrates is discussed.

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STRUCTURAL CHANGES ASSOCIATED WITH ADVANCING AGE IN THE THYROID GLAND OF THE FEMALE RAT WITH PARTICULAR REFERENCE TO ALTERATIONS IN THE CONNECTIVE TISSUE[1]

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TWO PLATES (TEN FIGURES)

In the last few years there have appeared numerous reports concerning structural changes that occur in association with advancing age in certain endocrine and reproductive organs. Of these organs the thyroid gland has perhaps been most extensively studied. Cooper ('25), Allara ('34), Dogliotti and Nizzi Nuti ('35), Rice ('38), and Hertzler ('39) studying human material; Loeb and Simpson ('38) working with the guinea pig; Smith and Starkey ('40), and Andrew ('41) using the mouse; and McCarrison ('17) studying the rat, have all described structural changes in the thyroid which were associated with the ageing process. Along with the other changes noted, an increase in the amount of connective tissue was generally found. Allara, the only one of the above investigators to use technical methods which differentiated reticulum from collagen, both of which are known to occur in the thyroid, has reported a coarsening of the reticular fibrils and a possible transformation of reticulum into collagen. These changes are known to accompany advancing age in many organs and tissues.

In this paper we wish to record observations of the structural changes which take place in the thyroid gland of the female rat as age advances. The most extensive observations were those made of the changes occurring in the connective tissues, and in this phase of the study, technical methods which permitted the differentiation of reticulum from collagen were employed. However, alterations which occurred in other parts of the gland, such as variations in size of the follicles or in the height of their lining epithelial cells, as well as variations in the staining reaction of the colloid content, were noted. In general these latter observations confirmed those of previous investigators.

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Since a knowledge of the general nature and distribution of the connective tissue stroma of the thyroid gland as well as the relation of the thyroid capillaries to the thyroid follicles is of fundamental importance as a basis for this study, a brief discussion of certain previous contributions concerning these subjects is relevant. It is well known that a connective tissue capsule encloses the thyroid gland, and that fibrous septa are distributed throughout the organ along the course of the blood vessels which penetrate the gland. Flint ('03) using digestion methods in studying the thyroid glands of humans, dogs, and monkeys, found that fine connective tissue fibrils formed a dense interlacing meshwork around the individual follicles; this meshwork was designated as a reticulated basement membrane. In addition to the interlacing meshwork immediately surrounding the follicles, he also found variable amounts of "interfollicular" connective tissue. In the human thyroid gland, most of the interfollicular tissue was arranged along the larger blood vessels, and in the dog such tissue was scant except in the neighborhood of such vessels. Somewhat later Livini ('09) described a meshwork of fine fibrils, which he thought were collagenous in nature, around the thyroid follicles. In the same year Major ('09), by means of injection methods, found a rich capillary meshwork around the thyroid follicles in man, in the cat, and in the dog.

In 1920 Corner, using the Bielschowsky-Maresch silver impregnation technique, found that in the thyroid of the rat there were strands of collagenous fibers along the course of the large blood vessels, but that between the follicles only reticular fibrils were present. These he thought were in the capillary endothelial cells themselves. Rhinehart ('30) later reported similar findings. He described a single layer of reticulum between the thyroid cells and vascular channels, and considered that the reticular fibrils were formed by, and were embedded in, the capillary endothelium.

In a very complete analysis of the human thyroid gland, Allara ('34) described collagenous and elastic fibers in the capsule. He described septa of the first, second, and third orders penetrating the gland. The septa of the first and second orders, which were collagenous and elastic in nature, divided the gland into lobules, but the lobulation was distinct only during the first year of life, and disappeared as age advanced. The septa of the third order (perifollicular septa) formed a collagenous meshwork about the thyroid follicles. Lying inside this meshwork was a net of fine reticular fibrils which were in direct contact with the thyroid epithelium and were also closely associated with reticular fibrils about the capillaries. Advancing age was associated with a coarsening of the reticular fibrils of this network and the apparent transformation of reticulum into collagen. In the capsule and the septa he found a marked increase of elastic tissue but no increase in the collagenous fibers. The cellularity of the stroma was greatly decreased.

Nonidez ('37) also described a fine reticular meshwork about the individual follicles in the thyroid of the cat. In the guinea pig Staudacher-Dalle Aste ('41a) reported that the connective tissue septa which penetrated the gland were gradually transformed into delicate laminae. Fibers from these laminae formed a relatively coarse meshwork about the individual follicles. In addition there was a meshwork of finer fibrils which was in direct contact with the thyroid cells. The same author ('41b) later studied the distribution of the capillaries about the thyroid follicles. He found that they formed a network surrounding the follicles, but that the thyroid cells were separated from the capillary endothelium by a meshwork of fine argyrophilic fibrils.

The above reports, considered as a whole, seem to indicate that in all the species studied there is a delicate mesh of reticular fibrils about the individual follicles. These fibrils separate the follicular epithelium from the network of perifollicular capillaries. In some species at least, there also seems to be a coarser and more irregular collagenous meshwork between many of the thyroid follicles. From the descriptions given, it seems most likely that these collagenous fibers are continuous both with the reticular meshwork and also with the collagenous elements of the connective tissue septa which accompany the blood vessels.

MATERIALS AND METHODS

The thyroid glands of ninety female rats distributed uniformly in the following age groups given in days, 10, 15, 30 to 50, 90, 180, 360, 500 to 700, and 720 to 884 were studied histologically. These glands were fixed in 10% formalin and cut serially at 6 microns. The sections were stained by Gomori's silver impregnation method for reticulum and collagen, Goldner's modification of Masson's trichrome method ('38), and with hematoxylin and eosin. By the silver technique reticulum is stained black and collagen old rose. Fibrillar material, transitional between reticulum and collagen, is stained purplish or blackish-red. By the trichrome method both reticulum and collagen are stained green, epithelium is reddish and colloid is either green or orange.

In order to establish quantitative bases for comparison of the age changes which occur in the thyroid gland, certain measurements were made. In the case of the connective tissue, the widths of the reticular fibrils and collagenous fibers in each group were measured with an ocular micrometer. In the case of the follicles, their relative size was determined. For this purpose a straight line was drawn on a circular plastic coverslip which was inserted into the ocular. The ocular was then adjusted so that the line on the coverslip was parallel to the shortest dimension of the section. The lumina of all follicles transected by this line were measured. An average measurement was thus obtained and this was used as an index of follicle size.

OBSERVATIONS

Before considering the changes associated with ageing, the general nature and distribution of the connective tissue of the thyroid gland of the rat should be described briefly. In rats of all ages this gland was covered by a collagenous capsule which was particularly prominent between the thyroid and parathyroid (fig. 9). Connective tissue septa followed the blood vessels into the thyroid tissue, and as the vessels divided and became smaller, there was a corresponding reduction in the amount of accompanying collagenous tissue. Although these septa divided the gland into irregular segments, definite lobulation was not noted.

Around the individual follicles a meshwork of reticular fibrils was found which was in close association with the thyroid capillaries. The fibrils of this meshwork were in contact with the capillary endothelium and separated this endothelium from the thyroid cells (figs. 1 and 6); thus the meshwork should be considered as pericapillary in nature. In our opinion, the reticular meshwork seen around the follicles was in reality the reticulum which formed the network around the perifollicular capillaries.

Thicker connective tissue fibers, apparently independent of the thyroid capillaries, were also found between the thyroid follicles, but they were less abundant. Except in the very youngest rats they were collagenous in nature, and became thickened, more conspicuous, and increased in number as age advanced (figs. 3, 10). Study of our material indicates that these thicker collagenous fibers were generally separated from the follicles by the pericapillary reticular meshwork. This does not exclude the possibility that in some instances thicker collagenous fibers may partially or completely surround the capillary and thus come in contact with the thyroid epithelium. In some instances at least these collagenous bundles, which we shall refer to as the interfollicular connective tissue, could be shown to be continuous with the collagenous septa which accompanied the ingrowing blood vessels, and we are inclined to the view that they represent outgrowths of these septa. These collagenous fibers also seemed to blend with the fine fibrils in the reticular meshwork about the follicles (fig. 4). The interfollicular connective tissue fibers described here are believed to correspond to the interfollicular tissue of Flint, to the pericapillary septa and possibly the septa of the second order of Allara, and to the coarse meshwork which Staudacher-Dalle Aste described lying superficially about the follicles. Our studies also confirm the generally accepted fact that reticulum is a pre-collagenous type of connective tissue and may give rise to collagen.

A differential staining of thyroid colloid with Mallory's connective tissue stain has been described by Hewer ('27) and Stein ('40), and both have correlated the staining reaction of the colloid with the activity of the gland. Thus in follicles in which there was structural evidence of functional activity, the colloid stained blue, while in inactive follicles it was orange-colored, and in some follicles a mixing of these colors was noted.

In these studies, a differential staining reaction of colloid was also noted. With Gomori's silver method, the colloid in the follicles which presented structural evidence of activity (as judged by the height of the epithelium) stained gray, while the colloid in the follicles with flattened epithelium took an old rose color. In numerous follicles a mixture of both gray and old rose colloid was observed, which gave either a mottled effect or a color intermediate between gray and rose. A similar contrasting reaction of the colloid to Masson's trichrome stain was observed; the colloid in the follicles judged to be functionally active stained light green, while that in the follicles with lower epithelium stained orange. A mixed coloration was frequently noted.

A close relationship between age and follicular size was noted. The follicles increased in size until the age of 45 to 50 days was reached. A temporary cessation of growth was noted in the 90-day-old rats, but beyond this period an increase in follicular size continued until the age of 180 days, when the average follicular size became more or less stabilized. Except for the temporary cessation of follicular growth in the 90-day-old rats, the course of growth of the follicles in the rat was quite similar to that found by Smith and Starkey ('40) in the mouse.

Definite changes correlated with advancing age were found in the connective tissue. In rats 10 to 15 days of age, the capsules of the glands were made up of loosely arranged, thin collagenous fibers or of fibers transitional between reticulum and collagen. Narrow collagenous fibers, or again, fibers which were transitional between reticulum and collagen and continuous with those found in the thin capsule, were found about the larger blood vessels. Around the individual follicles, a meshwork of fine reticular fibrils was found. Reference to figures 1 and 6 will indicate that the follicular cells were in contact with the thin reticular fibrils about the capillaries. Thus the thyroid cells were separated from the capillary endothelium by a very thin layer of connective tissue. Figure 2 shows a section cut through the peripheral area of a small follicle. A dense basket-like reticular meshwork about the follicle is seen, and beneath the meshwork thyroid cells can be observed. Although accurate measurements were impossible, it is estimated that the individual fibrils measured 0.3 to 0.5 microns in thickness. Occasionally, thicker interfollicular fibers were found. These were transitional between reticulum and collagen, and measured up to 2 microns in thickness. The largest follicles measured approximately 85 microns; generally, these were located near the periphery of the gland. The lining epithelium was in most instances cuboidal or low columnar. In sections stained with the silver impregnation technique, the colloid was almost entirely gray.

In the thyroid glands of rats 30 to 50 days of age, the width of the fibrils of the pericapillary reticulum was usually slightly greater than in the previous group. The capsules of these glands were generally slightly thicker and were collagenous in nature. The amount of connective tissue about the blood vessels was also slightly increased and was mostly collagenous. The interfollicular connective tissue fibers measured up to 5 microns in thickness, and were definitely collagenous in nature. Although there was marked variation, the thyroid follicles were on the whole definitely larger, the largest measuring about 175 microns. The thyroid epithelium was generally cuboidal, and the colloid stained gray, although old rose colloid was seen in some follicles.

In the rats 90, 180, and 360 days of age, progressive changes were noted both in the connective tissue elements and in other follicular structures. In all of these groups the fibrils of the reticular meshwork about the follicles measured about .6 microns in thickness. The interfollicular collagenous fibers became increasingly prominent in rats of these ages, increasing from a maximum thickness of 6 microns in 90-day-old rats to 8 microns in 360-day-old animals. Figure 4 shows the reticular meshwork at the extreme periphery of a follicle from a 395 day-old rat. It is cut slightly outside the follicular epithelium, but the endothelial cells of the capillaries in this region and the surrounding meshwork of reticulum are well demonstrated. Outside the reticular meshwork thicker collagenous fibers which make up the interfollicular tissue are seen. These blend directly into the reticular fibrils. Figures

5 and 7 show successive sections through the same follicle. Figure 7 is from the extreme periphery and shows the reticular meshwork on the surface of the follicle. Figure 5 shows the next section progressing toward the center of the follicle; it shows the meshwork cut obliquely. The next inward section in the series, which is not illustrated, appeared as an ordinary cross section similar to those in figure 10. There was a continued increase in the thickness of the collagenous thyroid capsule and the connective tissue partition between the thyroid and the parathyroid. The rather loosely arranged collagenous fibers found in the very young rats were replaced by thick collagen bundles in which the constituting fibrils were closely compacted. There was a continued increase in the amount of collagenous material around the large blood vessels.

There was a further increase in follicle size in the 180-day group, but beyond this period, the average of the follicle size became relatively stabilized. There was, however, great variation in size, and the largest follicles, which were generally located near the periphery of the gland, measured up to 225 microns. Associated with this increase in follicular size, there was a generalized decrease in the height of the follicular epithelium and a generalized change in the staining reaction of the colloid; the number of follicles with gray colloid decreased while those with old rose colloid increased. Many follicles were noted which contained both gray and rose-colored colloid. Generally, but not invariably, the gray colloid persisted in the smaller follicles in which cuboidal rather than flattened epithelium was found.

In the two oldest age groups, 500 to 700 days, and 701 days and older, the age changes reached a maximum. The reticular fibrils in the pericapillary meshwork about the follicles commonly measured up to 1 micron in thickness and occasionally more. There was not only an increase in the thickness of the individual fibrils, but the meshwork as a whole appeared to be made up of an increased number of fibrils. Both of these factors resulted in an increased density and irregularity of the reticular meshwork.

The bundles of interfollicular collagenous tissue were quite abundant; the thickest of these measured up to 10 microns in diameter (figs. 3, 10). Figure 8 is taken from near the periphery of a follicle of a rat 793 days old. Although reticulum and collagen are not well differentiated in this photograph, the dense meshwork shown on the surface of this follicle consisted of reticular fibrils and more superficial coarse collagenous fibers. The capsules and the partition between the thyroid and the parathyroid reached maximum thickness, and were made up of dense collagenous fibers.

Although there was no generalized increase in follicular size, some very large follicles, measuring up to 350 microns, were observed. As a rule the larger follicles had flattened epithelium and rose-colored colloid, while the smaller follicles had higher epithelium and gray colloid. However, numerous transitional stages in the staining of the colloid were noted.

DISCUSSION

Previously we have studied the effects of advancing age on the connective tissue of the uteri (Wolfe et al., '42) and the anterior pituitary glands (Lansing and Wolfe, '42) of the same group of female rats from which the thyroids considered in the present study were taken. Although the amount and distribution of the connective tissue is different in the three organs, the fundamental response of the fibrillar material to advancing age seems to be the same. The degree of this response and the rapidity with which it progresses are apparently characteristic for each separate organ.

In the uterus a thickening of the reticular fibrils of the endometrium and a transformation into collagen, which occurs when the fiber is less than 1 micron in thickness, is noted in rats between 10 and 15 days of age. The transformation of endometrial reticulum into collagen is largely completed early (90 days) in the life span. Throughout the remainder of life there is a continued transformation of the remaining reticulum into collagen and a continued thickening of the collagenous fibers, which sometimes measure up to 30 microns in width.

In the anterior lobe of the pituitary gland, practically all the fibrillar tissue is reticular, and is found in the walls of the sinusoids. The reticular fibrils enlarge slowly and progressively throughout life, and reach a maximum thickness of 2.5 microns near the end of the life span. They show little tendency to transform into collagen, although fibrils transitional between reticulum and collagen were occasionally noted.

In the thyroid, the reticular fibrils in the pericapillary meshwork around the thyroid follicles thicken slowly throughout life, but never reach a maximum diameter over 1.2 microns even in very old rats. As in the case of the sinusoidal reticulum of the anterior lobe of the pituitary gland they show little tendency to transform into collagen. However, the number of fibrils in the meshwork apparently increases with age; this numerical increase, combined with the thickening of the individual fibrils, results in a denser, a thicker, and a more irregular meshwork.

The changes in the so-called interfollicular fibers and the connective tissue found along the course of the blood vessels are more conspicuous. In the 10- and 15-day-old rats these fibers measure up to 2 microns in thickness and are usually transitional between reticulum and collagen. As age advances, they rapidly become purely collagenous and increase markedly in number and size. In old rats the interfollicular collagenous tissue is quite abundant and individual fibers measure up to 10 microns, and sometimes more, in thickness.

Thus it is seen that in all three of these organs advancing age is correlated with a thickening of the reticular fibrils. In certain instances they are transformed into collagenous fibers, and throughout the life span there is a continued increase in the amount of collagenous tissue. In other instances the transformation of reticulum into collagen does not generally occur. It should be emphasized, however, that all reticulum probably has the capacity to transform into collagen. In rare instances it has been observed that the sinusoidal reticulum in the anterior lobe and the reticulum in the meshwork about the thyroid follicles were replaced by collagen.

We have pointed out previously (Lansing and Wolfe, '42) that the ageing pattern of the connective tissue of an organ is probably determined by the function which the fibers serve in the organ. It seems probable that the chief function of the fibrous tissue in the uterine endometrium and the interfollicular and septal fibrous tissue of the thyroid is to form a supporting framework. These fibers become collagenous early in life and increase markedly in size and number. The fibrils of the pericapillary meshwork around the thyroid follicles and the sinusoidal reticulum of the anterior lobe lie directly in contact with the glandular cells. Although they undoubtedly support these cells, they also must take some part in the interchange of nutritive materials and glandular secretion. The reticular fibrils thicken slowly and progressively throughout life, and transform only infrequently into collagen. However, the relationship between the apparent function of connective tissue fibers and the nature of their response to advancing age is obscure and deserving of more study.

The observations in this paper indicate that the ageing changes which occur in the connective tissue of the thyroid gland and those which occur in the thyroid epithelium occur simultaneously, and furthermore, that the structural changes begin early in life and continue throughout the life span. Although there is almost certainly a close relationship between the reaction of these two elements of the gland to advancing age, the nature of the association is not clear. For instance, it is not known whether age changes occurring in the epithelium and in the connective tissue are independent of each other, or whether the alterations which occur in one component are the cause or the result of primary alterations in the other component. The authors are inclined to believe that the changes which occur in the connective tissue of the thyroid as age advances are secondary to alterations occuring in the epithelium. In spite of our lack of knowledge concerning the possible relation between age changes in the thyroid cells and in the stroma, it seems clear that the gradual decrease in the height of the thyroid epithelium and the increase in follicular size and accumulation of follicular colloid, which occur as age increases, are associated with an increase in fibrous tissue. Since the changes which occur in the thyroid epithelium and colloid are generally considered as evidence of decreased functional activity, at least a hypothetical relationship between an increase in the amount and density of fibrous tissues and a decreased functional activity may be predicated. It should be pointed out that Allara has already suggested a relationship between functional activity of the follicles and the distribution of connective tissue on their surfaces. Around the small follicles which he designated as hyperfunctional, he found an abundance of fine reticular fibrils. In contrast, he found a lesser number of reticular fibrils about the large follicles, considered to be hypofunctional, but they were much coarser. It seems quite clear that this possible relationship between functional activity on the part of the thyroid follicles and the nature of the perifollicular connective tissue should be the subject of further investigation.

Bruger and Rosenkrantz ('42) have recently suggested a relationship between the structural changes associated with ageing in the human thyroid and the lowered basal metabolism which occurs in man as age advances. The general problem of the relation of the thyroid to ageing phenomena has been discussed in detail by Carlson ('42).

SUMMARY

The thyroid glands of ninety virgin female rats killed between the ages of 10 and 884 days were studied. Definite changes which could be correlated with advancing age were found in the connective tissue, the thyroid epithelium, the follicular colloid, and the size of the follicles.

The rat thyroid is surrounded by a connective tissue capsule, and strands of connective tissue accompany the blood vessels into the gland, forming septa. In very young rats (10 days) the capsule and the septa contain either thin collagenous fibers or fibers transitional between reticulum and collagen. As age advances, there is a transformation

of the transitional type into collagen, and a continued thickening and increase in the number of collagenous fibers continues throughout life.

The thyroid follicles are surrounded by a meshwork of pericapillary reticular fibrils which separate the epithelium from the capillaries. As age advances, these reticular fibrils gradually become coarser and more numerous, resulting in a denser and more irregular meshwork about the follicles. These fibrils rarely change into collagen.

In addition to the reticular meshwork about the follicles, coarse strands of connective tissue (interfollicular connective tissue) probably continuous with the ingrowing septa, occur less frequently between the thyroid follicles. In very young rats these fibers are transitional between reticulum and collagen. As age advances, they are transformed into collagen and increase markedly in width and number.

In young rats the thyroid follicles are small, are lined by cuboidal or low columnar epithelium, and contain colloid which stains gray with silver impregnation procedures. With advancing age there is an increase in the size of the follicles, a decrease in the height of the thyroid epithelium and a shift in the staining reaction of the colloid from gray to a rose color.

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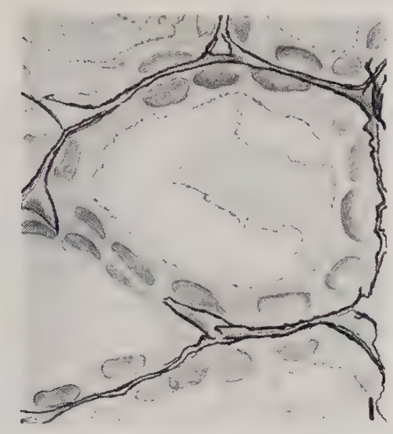

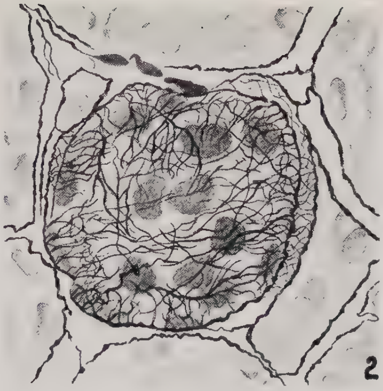

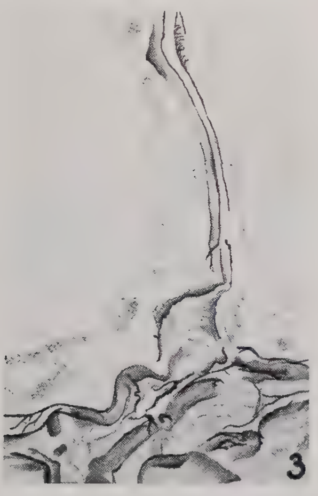

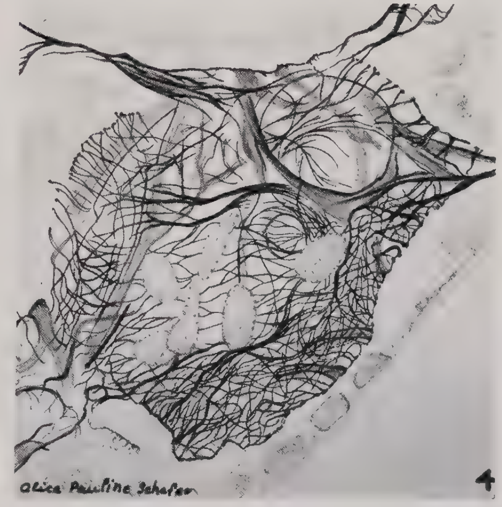

(All tissues fixed in 10% neutral formalin and stained by the Gomori technique. All magnifications are approximately × 1850).

1 Section near the center of a thyroid follicle of a 15-day-old rat. Note the pericapillary reticulum in contact with the thyroid epithelium.

2 Section cut near the periphery of a thyroid follicle of a 10-day-old rat. A reticular meshwork is seen on the surface of the follicle and below this meshwork the thyroid cells can be seen. The capillary to the right of the follicle is cut so that the reticular meshwork on the floor of the capillary is visible. Three endothelial cells are seen in the capillary above the follicle.

3 Sections near the center of two thyroid follicles of a 785-day-old rat. Thick collagenous fibers and a meshwork of fine collagenous fibers are seen to lie between the follicles: these constitute the so-called interfollicular connective tissue.

4 This section is from a 395–day-old rat. It is cut at the extreme end of a thyroid follicle so that the reticular meshwork on the surface of the follicle and endothelial cells in the perifollicular capillaries are seen. Underneath the reticular meshwork coarser collagenous fibers are observed. It is seen that these blend directly into the reticulum.

PLATE 2

DESCRIPTION OF FIGURES [2]

(All tissues were fixed in 10% neutral formalin and stained by the Gomori technique).

5 Section near the periphery of a thyroid follicle of a 395–day-old rat so that an oblique section of the reticular meshwork about the follicle is shown. Figure 7 shows the succeeding section, and the reticular meshwork on the surface of the follicle can be seen. × 880.

6 Section showing cross section of several follicles of a 15-day-old rat. The reticulum about the follicle is shown. × 690.

7 Section cut at the extreme periphery of a thyroid follicle of a 395-day-old rat, showing the reticular meshwork on the surface of the follicle. See description of figure 5. × 880.

8 Section cut at the extreme periphery of a thyroid follicle of a 793-day-old rat. Although reticulum and collagen are not differentiated in this photograph, the dense meshwork on the surface of this follicle consisted of reticular fibrils and more superficially, dense collagenous fibers. × 880.

9 Section showing the junctions of the thyroid and parathyroid of a 10-day-old rat. The fibrous capsule between the two is made up of thin and diffuse collagenous fibers. × 274.

10 Section through several thyroid follicles of a 785-day-old rat. Note the thick collagenous fibers between the follicles. × 690.

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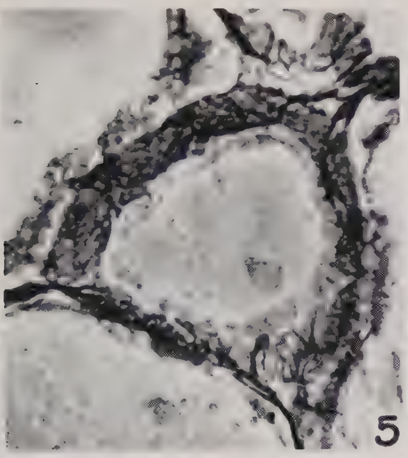
[2] We wish to thank Mr. E. G. Moore, medical school photographer, for making the photomicrographs.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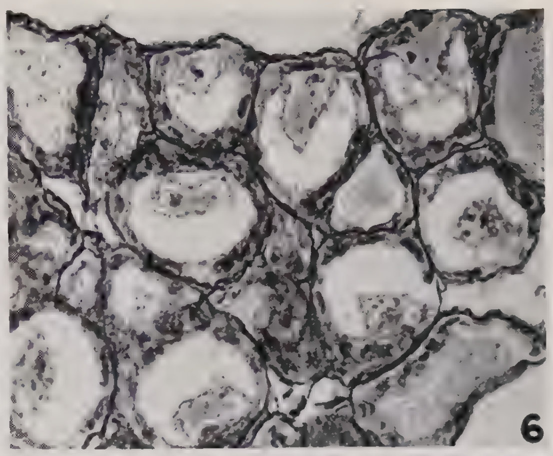
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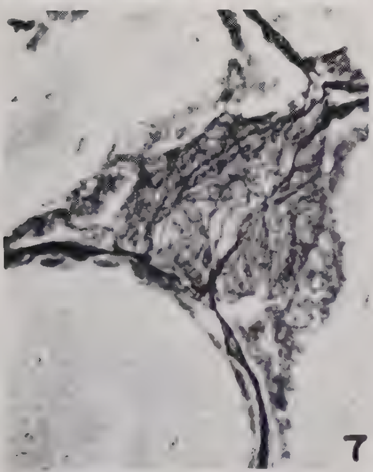
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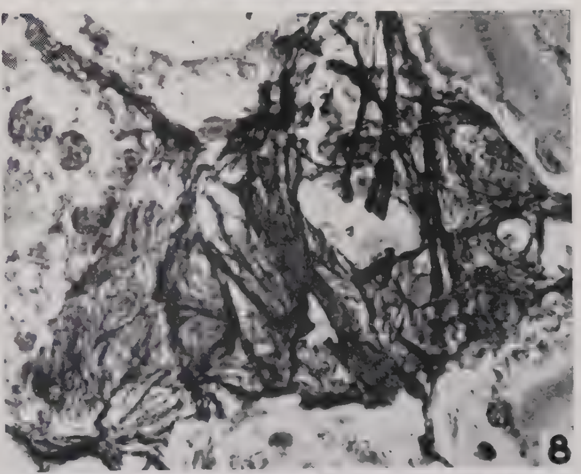
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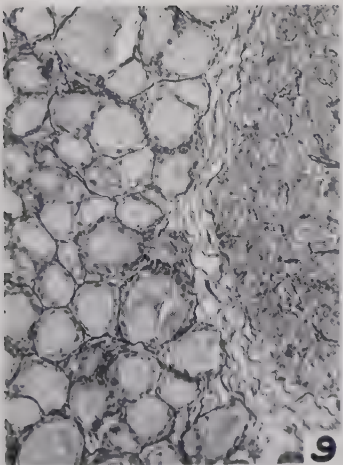
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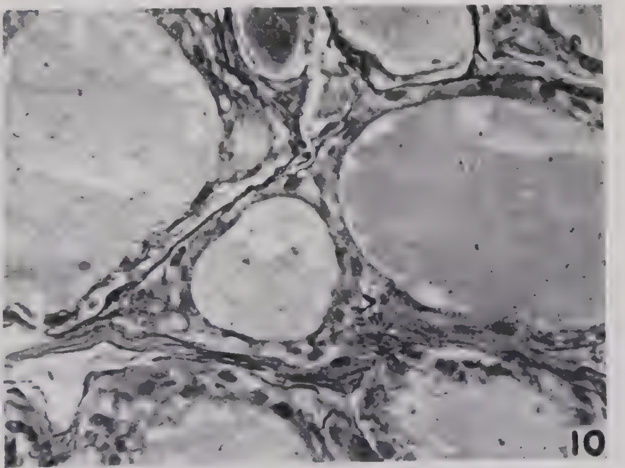
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BOOK REVIEW

EDWARD TYSON, M. D., F. R. S. 1650–1708 AND THE RISE OF HUMAN AND COMPARATIVE ANATOMY IN ENGLAND — A Study in the History of Science by M. F. ASHLEY MONTAGU. Memoirs of the American Philosophical Society, Vol. XX, 1943. xxiix 488 pp.

In Europe the human anatomist often indulges in antiquarian research as an avocation; he may take up the study of fossil man, as did Sir Arthur Keith, or the prehistoric diffusion of cultural traits, as did Sir Grafton Elliot Smith. Prof. Ashley Montagu is exceptionally versatile; he is not only a human anatomist, but also a physical anthropologist, a social anthropologist, and, now, a historian of science. He is admirably equipped for the last named function, not only because of his breadth of interests and his formidable erudition, but also because of his literary ability. He writes exceedingly well.

Ashley Montagu's enthusiasm for Edward Tyson, the subject of the present book, goes back to 1923, when as a student, he read on the first page of Duckworth's Morphology and Anthropology a very brief account of Tyson's principal contribution to physical anthropology. This reviewer read the same account in 1910, when he was also a student, but his knowledge of Edward Tyson was not appreciably enlarged until, within the past few weeks, he availed himself of the fruits of Ashley Montagu's labors. This merely goes to show that some men have a superior capacity for inspiration. Ashley Montagu encountered Tyson in Duckworth and inspired; I merely inhaled and exhaled forthwith. I fall so far short of the gifts of Ashley Montagu that, as a reviewer, I cannot do justice to his fine book on Tyson, which is easier for me to admire than to read.

Tyson was a stupendous fellow who seems to have been practically the founder of the modern method of comparative anatomy, as well as the first person to carry out a detailed comparison of the anatomy of an anthropoid ape with that of a man. Says Ashley Montagu (p. 417):

> "It will be seen that Tyson's principal contribution to the development of Western thought has been in focussing attention upon the relationship of man to the apes. The first cogent suggestion that such a relationship was probable came from Tyson, and subsequent interest in the idea was to a large extent initiated, and as we have seen was for a long time supported, by his original work. It would be difficult to overestimate the importance and far-reaching consequences of the revolution in our ideas concerning the descent of man thus initiated. It is clear that Tyson's part in producing that revolution entitles him to a high place among the creators of our present culture."

The book gives as full an account of Tyson's life as the author could compile from extensive reading and no little biographical research. It describes in detail and appraises Tyson's published scientific works, which were extraordinary in their scope and, without exception, first-rate. All of this material about Tyson is made by Ashley Montagu the vehicle for carrying a history of the development of comparative anatomy in England. The work is beautifully and fully illustrated and the numerous footnotes are stuffed with interesting facts and valuable observations derived from the author's wide reading in the history of science and from his active career as an anatomist.

E. A. HOOTON,
Harvard University

INTRAUTERINE DISTRIBUTION OF OVA IN THE RABBIT[1]

J. E. MARKEE
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ONE FIGURE

The factors responsible for the intrauterine transport and distribution of ova are still incompletely understood. Wislocki ('24) reports that active myometrial contractions occur in strips of uterine muscle from the sow on the day of ovulation and for 3 days thereafter and that the activity decreases between the third and seventh day and has practically disappeared by the fifteenth day. Keye ('23) and Corner ('23) report similar observations and Wislocki ('24) suggests that myometrial contractions may be responsible for the distribution of ova within the uterus. However, on the basis of a study in which he used an intrauterine balloon connected to a Brodie bellows, Reynolds ('37) concludes that ". . . in the rabbit, which ovulates normally about ten hours following coitus or other suitable stimulus, the (uterine) contractions nearly disappear some five hours or more prior to ovulation and this state of uterine quiescence remains unchanged for nearly the duration of a pseudopregnancy or pregnancy." Reynolds and Friedman ('30) and Sager and Leonard ('36) arrive at similar conclusions. If these conclusions are valid, the transportation of ova and their uniform distribution within the uterine horns cannot be accomplished by myometrial contractions alone.

To determine more precisely the factor, or factors, involved in the uniform intrauterine distribution of ova in the rabbit, the problem has been investigated by two methods: eggs from another species have been introduced into the uterus experimentally and the extent and uniformity of their distribution recorded in estrous and pseudopregnant rabbits, and uterine contractions have been studied and photographed through a glass window in the abdominal wall.

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[1] Aided by grant from Sex Division, National Research Council.

PROCEDURE

The preliminary preparation of the rabbits consisted merely of transection of the spinal cord at the fifth or sixth thoracic segment. Three to 6 days later the abdomen was incised aseptically and an opening surrounded by a purse string suture was made in the antimesometrial border of each uterine horn about 1 cm. from its tubal end. (In six of the rabbits this part of the procedure was changed and the opening was made near the cervical end of the uterine horns). Approximately 100 stained sea urchin ova [2] in 0.1 cc. of normal saline were introduced through the opening into the uterus by means of a syringe, the uterine horn having been compressed 2.0 cm. distal to the opening lest the ova be forced along the horn during the injection. Two hours later the abdomen was reopened and the uterine lumina divided into three equal compartments by placing two ligatures around each uterine horn to prevent mechanical displacement of ova during the removal and subsequent manipulation of the uterus. The uterine compartments were opened successively and the location of the ova recorded with the aid of a long-arm binocular dissecting microscope and camera lucida. This procedure was carried out on four groups of rabbits; (1) during estrus, (2) at the time of ovulation, (3) on the fifth day, and (4) on the tenth day after ovulation.

RESULTS

The distribution of the sea urchin eggs, celloidin balls, and glass beads injected into the tubal ends of twelve uterine horns of estrous rabbits is shown in figure 1. In nine of the ten uterine horns in which the sea urchin eggs were injected near the tubal end the ova were uniformly distributed, the only inconclusive experiment being the right horn of no. 41 in which only disintegrated portions of the sea urchin eggs were found. The six experiments in which the sea urchin ova were injected into the cervical ends of the horns were not included in figure 1 since the ova were distributed as evenly as in the ten experiments in which the ova were injected into the tubal end of the uterine horn. Of the forty celloidin balls injected into the right horn of no. 446, only twenty-three near the center of the horn could be identified. None of the glass beads injected into the right horn of no. 445 could be located.

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[2] In a series of preliminary experiments, ova from a number of other species and small foreign bodies such as glass beads, celloidin balls and other particulate matter proved unsatisfactory. However, sea urchin ova, suggested by Dr. D. M. Whitaker, are satisfactory for the purpose of this experiment since they do not clump, are readily stained by Wright's stain, and are similar in size, shape, and consistency to rabbit ova.

Sea urchin eggs were injected into twelve uterine horns at about the time of ovulation; i.e., 12 hours after the administration of chorionic gonadotrope. In three uterine horns (fig. 1, right and left horns of EB and right horn EA) the ova were distributed as evenly as in the estrous rabbits. In seven uterine horns the ova were distributed almost as evenly, in five of them the ova were slightly less concentrated in the cervical half. The rabbit DF probably does not belong in this series of experiments since the ovaries contained, in addition to the freshly ruptured follicles, seven corpora lutea judged to be approximately 10 days old. These corpora were almost certainly still functional and probably explain the fact that all of the sea urchin ova had remained in the tubal half of the left horn and that only five had reached the cervical half of the right horn.

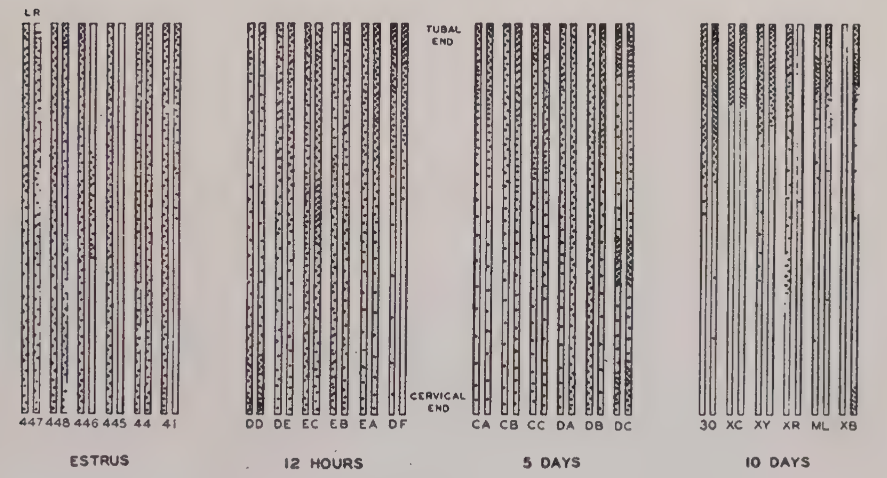
GRAPHIC REPRESENTATION OF DISTRIBUTION OF OVA (SEA URCHIN) IN RABBIT UTERI

Figure 1

In six rabbits, sea urchin ova were injected into the tubal ends of the uterine horns 5 days after the induction of ovulation. The eggs were evenly distributed throughout two of the uterine horns (right horn of DA and left horn of DB) and in eight the eggs were evenly distributed save for a slightly lower concentration near the cervical ends of the horns. In one rabbit (DC) in which no progestational reaction could be detected in sections of the uterus, although the sectioned corpora lutea appeared nearly normal, the ova were concentrated near the middle and ends of the horns.

The sea urchin eggs injected on the tenth day after induced ovulation were very poorly distributed in all of the twelve uterine horns, in striking contrast to the even distribution in the preceding three groups. Only a few of the eggs injected at this time traversed half of the length of the uterine lumen, the others remaining near the injection site. To test the possibility that better distribution might occur during a longer interval, sea urchin ova were left in the uterine horns of three similar rabbits for 24 hours. Since about one-third of the eggs could not be identified and many of the others were badly fragmented, the results were not included in figure 1. However, the identifiable intact eggs and fragments were found only in the tubal half of the uterine lumen, most of them near the site of injection.

Recently a technique has been developed by which it is possible to sew a divisible glass window into the abdominal wall of a rabbit. Observations may be made through such windows for 75 days or longer without reanesthetizing the rabbit. Through such windows daily observations were made on uterine contractions throughout the first 12 days of pseudopregnancy.

During estrus and for 5 days after ovulation, three types of contractions may be observed: local, peristaltic and anti-peristaltic contractions. The local contractions persist for about 10 seconds and do not progress in either direction. When most intense they obliterate the lumen thus forcing the uterine contents away from the constricted region for the duration of the contraction. This type occurs most frequently throughout pseudopregnancy and is often the only type observed, especially when the uterus is relatively quiescent. The peristaltic contractions usually begin at the extreme tubal end of the horn, pass slowly along it and reach the cervical end in about 60 to 90 seconds. Any fluid that may be present is sometimes driven ahead of the contraction and the uterus appears relaxed and collapsed behind the contraction. As the peristaltic wave approaches the cervical segment it appears distended and stretched. As an apparently single terminal event, the fluid is forcefully and rapidly propelled back toward the tubal end, seemingly by the elastic recoil of the stretched cervical segment. It does not appear that there is any active contraction of the cervical segment at this time. Antiperistaltic contraction usually begins near the cervical end of the horn and passes along it toward the tubal end, the rate of progression approximating that of the peristaltic ones. Save at about the time of ovulation it is observed less frequently and appears less forceful. This type of contraction usually drives a smaller amount of fluid ahead of it. Those antipersitaltic contractions that traverse nearly the full length of the horn cause less distension of the tubal end. The elastic recoil propels the smaller volume of fluid less forcefully toward, and seldom to, the cervical region.

The frequency, forcefulness, and rate of progression of the peristaltic contractions decrease somewhat a few hours before ovulation occurs. On the other hand, the antiperistaltic contractions occur more frequently and the augmented fluid is propelled toward either the cervical or tubal end of the apparently more relaxed horn at about 60-second intervals during the hours following mating.

During the 5 days following ovulation the frequency of the antiperistaltic contractions decrease and there may be a further decrease in the frequency and forcefulness of the peristaltic waves. However, an even more noticeable change occurs for both types of concentrations traverse a decreasing proportion of the length of the horns. The shortening of the excursion is especially noticeable between the fifth and seventh day of pregnancy or pseudopregnancy and thereafter few of the contractions traverse as much as one-quarter of the length of the horns.

The nice coordination of the uterine contractions is easily disturbed by fright or adrenalin or undue pressure applied via the neighboring viscera or abdominal wall. A recording balloon inserted through the vagina and cervix causes almost complete disappearance of the contractions that were occurring before the beginning of this procedure. The contractions recorded by such a balloon are almost exclusively a different type of contraction. These new contractions appear to be the results of the attempts of the uterus to dislodge the balloon and unless these new contractions are opposed they soon force the balloon out of the uterus.

DISCUSSION

Under normal conditions it is conceivable that the distribution of the rabbit's own ova might be affected by contractions of the abdominal muscles, gravitational force, ciliary action, intestinal peristalsis, spontaneous generalized body movements, or other factors. However, transection of the spinal cord at the sixth thoracic segment practically eliminates any possible effects due to changes in intra-abdominal pressure resulting from contractions of the abdominal muscles or local or generalized contractions of the body musculature. Furthermore, intestinal peristalsis can hardly compress the thick-walled uterus sufficiently to transport ova, especially in an animal whose abdomen is flaccid. Ciliary action can be eliminated as a possible explanation of these findings since sea urchin ova injected into the cervical end of the six uterine horns were distributed as evenly as those injected into the tubal end. Neither can ciliary action explain internal migration of ova in the sow (Corner, '21) and cat (Markee and Hinsey, '33) since the cilia, if present, could not carry the ova down one horn and up the other. If gravitational force were responsible for the even distribution of ova in three of the groups, it should have produced equally good distribution in the fourth. Furthermore, distribution was equally good in some rabbits (44 and 448) which were never moved out of a horizontal position throughout the experiment. If predetermined implantation sites do exist, the distribution of sea urchin eggs was not affected by them, since the eggs were not concentrated in the vicinity of hypothetical sites.

Although any one of the three types of uterine contractions would appear to be capable of distributing either the sea urchin eggs or the rabbit's own ova, it appears likely that the peristaltic contractions are primarily involved. When observed through the glass window, the peristaltic contractions seem most effectively to distribute uterine contents, and other experiments indicate their superior effectiveness. Thus the rapid transport of sperm, which in the rat may reach the tubal end of the uterine lumen in 60 seconds (Hartman and Ball, '30), might be due to either peristaltic or antiperistaltic contractions. However, in his study of intrauterine transportation of sperm, Rossman ('37) described recoils of uterine contents after peristaltic contractions but no antiperistaltic contractions in the uteri of rats examined immediately after coitus. In addition, during coitus in the dog, the rhythmic forceful ejection of sperm for a distance up to 12 cm. out of the upper end of a uterine horn projecting through a fistula (Evans, '33) can be explained by the recoil after the cervical segment has been distended by a peristaltic contraction but not by the slow milking action of an antiperistaltic contraction. Furthermore, intrauterine migration of ova, which has been observed only in species possessing a large common portion of the uterus (Markee and Hinsey, '33) as in the sow and cat can be accomplished by peristaltic contractions and recoils even if the contractions occur simultaneously in the two uterine horns.

The peristaltic contractions and recoil can best explain the rapid and even distribution of sea urchin eggs injected during estrus. The slight decrease in the evenness of the distribution of the eggs injected at the time of ovulation may be due to the combined effect of the decreased intensity and frequency of the peristaltic contractions and the increased frequency of the antiperistaltic contractions. The poorer distribution of the eggs injected on the fifth day after ovulation is apparently due to the continued decrease in the intenstiy and frequency of the peristaltic contractions, the reduced frequency of the antiperistaltic contractions, and the failure of both to traverse the entire length of the horn. The virtual lack of distribution by the tenth day after ovulation may be attributed to a much greater reduction (not disappearance) of uterine motility and especially to the greater decrease in the excursion of both types of contractions.

Our observations on the persistance of uterine contractions after ovulation are in agreement with those of Wislocki ('24), Keye ('23), and Corner ('23) who found active myometrial contractions in the sow until the third to seventh day after ovulation, and these experiments on the distribution of the sea urchin eggs lend the first experimental support to their suggestion that myometrial contractions may account for the transportation and spacing of ova within the uterus.

These findings are also in complete agreement with Hartman's ('32) statement that ". . . in the uniparous monkey — in which the implantation site is fairly definitely fixed . . . the contractions are calculated to hold the ovum in a given region, as in an eddy, near the middle of the fundus (Ivy, Hartman and Koff, '31); in bicornuate uteri the end result is the spacing of the eggs . . ." That the uterine musculature is responsible for the spacing of the eggs was shown by Nicholas (unpublished) in an ingenious experiment. "For a section of the uterine horn in the rat he substituted a section of the duodenum, which healed in place. Eggs became implanted in the uterine tissue at the ends as well as in the interpolated intestinal tissue" (Hartman, '32).

The discrepancy between the findings implicating myometrial contractions as the cause of intrauterine distribution of ova, and Reynolds' ('37) description of the virtual disappearance of uterine contractions even before ovulation, may arise from the nature of the recording apparatus he used, for in his description of the technique Reynolds writes ". . . one might raise the objection that even the contractions recorded by this method are artifacts insofar as they are provoked by a foreign body, namely the balloon . . ." The question of whether the contractions recorded by this technique are normal contractions, as Reynolds subsequently assumed, or are instead primarily the attempts of the uterus to dislodge the balloon can be determined only by further investigation. In any case, uterine contractions were observed and the ova were distributed at a time when very few contractions are recorded by the intrauterine balloon technique.

SUMMARY

1. Sea urchin ova were injected directly into the uterine lumen in four groups of spinal rabbits; during estrus, at the time of ovulation, and 5 days and 10 days later. The ova were distributed most evenly in the estrous rabbits, nearly as evenly in the ovulation group, fairly evenly in the 5-day group, but were not distributed in the 10-day group.

2. Such factors as the contraction of abdominal muscles, gravitational force, ciliary action, intestinal peristalsis and generalized body movements appear to have been eliminated as causative factors in the distribution of the ova.

3. It appears that the ova were distributed by the contractions of the uterine muscle and this assumption is supported by direct observations of uterine contractions through a glass window in the abdominal wall.

4. If the observed myometrial contractions distributed the ova, the myometrial contractions were nearly as effective at the time of ovulation as during estrus and the effectiveness of the contractions continued until the fifth day after ovulation but decreased markedly between the fifth and tenth days after ovulation.

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ANTERIOR PITUITARY-THYROID RELATIONSHIPS IN THE FOWL

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TWELVE FIGURES

This paper is a study of the pituitary-thyroid relationships in the domestic fowl, primarily from the point of view of the cytology of the anterior pituitary following, (a) thyroidectomy, (b) thyroxin injections in normal male chicks, (c) thyroxin injections in thyroidectomized birds, (d) thyroidectomy in capons, (e) thyroxin injections in capons, (f) thyroxin and testosterone injections in capons. Pituitaries of dwarfs with retarded thyroid development have also been studied.

The experimental part of the work was done by Eric Simmons and W. R. Breneman and to them my thanks are due.

Thyroidectomy. Brief reviews of the effects of thyroidectomy on the anterior pituitary have been published by Severinghaus ('37, '38 and '39). In these papers the author stated five main conclusions. 1. A marked loss of acidophilia due to granule depletion reaches its height at about 40 days (rat) when it may result in a complete loss of acidophiles. The acidophiles return to an inactive chromophobe state. 2. The basophiles are greatly increased in number, arising from chromophobes of the basophilic line. 3. Less than a week after thyroidectomy, a marked increase of granular basophiles is obvious. From that time on, vacuolation of the basophiles begins and proceeds rapidly. 4. These basophilic changes are believed to be of the same regressive character as the vacuolation of the basophiles after castration, but the onset of vacuolation is earlier, and the speed and degree of vacuolation are far greater than in the castrate hypophysis. The hypophysis of the early thyroidectomized rat resembles that of a long term castrate. These modified cells, derived from the basophiles by Severinghaus, are commonly known as "thyroidectomy cells". 5. The acidophiles secrete the thyrotropic hormone. A sixth general conclusion of minor significance but one stated by many authors is that the pituitary becomes enlarged following thyroidectomy.

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[1] Contribution no. 326.

Not all authors are in agreement with these conclusions. Some of the differences are probably due to differences between species but even in the rat where most studies have centered, variations as to observations and conclusions are found. No attempt will be made to review the literature but a few citations follow to illustrate the confusion which now exists.

Guyer ('37) while describing vacuolation of basophilic cells following thyroidectomy, interprets these changes as degenerative in character in contrast to those following castration which he thinks are secretory. Zwecker et al. ('35) described the vacuolation of the basophiles but expressed the opinion that the vacuolated basophiles (thyroidectomy cells) are not castration cells and that they secrete the thyrotropic hormone. Zwecker ('37) believed she was able to distinguish morphological differences between thyroidectomy and castration cells. Bryant ('30) interpreted the vacuolated cells as enlarged chromophobes. Houssay et al. ('32) reported no increase in the thyroptropic potency of the rat pituitary following thyroidectomy and Smelser ('34) confirmed this conclusion. On the other hand Chen and Van Dyke ('36) reported an increase in the female rabbit but not in the male. It would seem that an increase in the thyrotropic content of the pituitary might be expected if the thyroidectomy cells secrete the thyrotropic hormone and a decrease if the acidophiles perform that function since thyroidectomy cells are additions and since the acidophiles seem to regress. Smith and Engle ('30) concluded that pituitaries from controls and thyroidectomized rats produced about the same results as to gonadotropic hormones, indicating that there is no marked disturbance in the output of these hormones. Such a conclusion is inconsistent, however, with the results of Winchester ('39), and Taylor and Burmester ('40), who found that egg production in the domestic fowl is markedly lowered following either complete or partial thyroidectomy; also with the results of Benoit and Aaron ('34) who reported a decrease in size of the testes of White Leghorn fowls following thyroidectomy. It seems clear that in the fowl there is a disturbance of normal ovarian and testicular function and that these disturbances may be due to a decrease in the secretion of gonadotropic hormones. On the other hand thyroidectomy may effect the normal metabolic activities to such an extent that gonad activity is reduced.

My study has been limited, for the most part, to White Leghorn male chicks varying in age from 40 to 82 days. The age at which thyroidectomy was performed varied from 3 to 36 days. The greatest changes occurred following early operations, but differences between 3 and 20 days were not marked.

Thyroidectomy in the fowl is followed by pronounced pituitary changes, most of which are different from those described in mammals; also by retarded body, comb and gonad growth. As an illustration of these gross changes the average body, comb and gonad weights of three 82-day-old chicks thyroidectomized at 4 days were 236 gm., 27 mg. and 55 mg. respectively.

Instead of the pituitary increasing in size following thyroidectomy as reported by Severinghaus in the rat, by Brant and by Van Dyke and Chen in the rabbit, the chick pituitary is reduced. When the areas [2] of longitudinal sections through the middle of the pituitaries were compared, the 40-day controls measured 5774, the 40-day chicks thyroidectomized from 3 to 10 days, 3977. The body weights of these two groups were respectively 337.7 and 125 gm.; the gonad weights 159 and 39.6 mg.; and the comb weights 2875 and 27.07 mg. It would have been better to have weighed the pituitaries but the least delay meant poor fixation and good fixation was more important than accurate comparisons of pituitary sizes. Even by the method used it is evident that the pituitary size of thyroidectomized chicks is less than in controls, but in comparison to body weight the size is as large or slightly larger than in controls. As will be pointed out later the essential differences between the pituitaries are cellular. The gonad and comb weights, however, are smaller in thyroidectomized chicks, even in comparison to body size. These low weights may be explained by the undeveloped state of the basophiles in thyroidectomized chicks. In the case of the comb, however, Simmons (unpublished data) has found that growth is blocked in the absence of the thyroid even though the sex hormone is present in injected form.

Perhaps the most controversial questions as to the effects of thyroidectomy pertain to the ''thyroidectomy cells'', which for brevity I shall call ''T cells''. Do they arise from basophiles, acidophiles, or chromophobes? Are they similar to or identical with the enlarged basophiles or castration cells which follow gonadectomy? Are they secretory and if so, do they secrete a gonadotropic hormone or do they secrete the thyrotropic hormone?

To enable the reader to better understand my description of what occurs in the anterior pituitary of the fowl following throidectomy, a few statements about the pituitaries of controls seem desirable. At the time of hatching and for the first 10 days the acidophiles and chromophobes are the dominant cell types in White Leghorn chicks. The acidophiles are predominantly in the posterior half of the gland and the chromophobes in the anterior half, although chromophobes are distributed throughout. The peripheral chromophobes at the anterior end and those on the ventral side of the anterior half as well as the cell cords retain their embryonic character. The cytoplasm, although containing no secretory granules stains reddish in contrast to the greenish color of the more centrally located chromophobes. The basophiles are evident at 10 days of age in the White Leghorn chick and are first observed in the mid-juxta neural or mid-dorsal region. Later they may be found anywhere throughout the pituitary but for the most part are aggregated in the middle and posterior parts of the pituitary. They are seldom found in the peripheral region of the anterior end.

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[2] For comparing gland size, outlines of median sagittal sections were drawn with a camera lucida at an enlargement of 58 diameters. These drawings were then traced with a planimeter to get the areas in sq. mm. The size given is the mean for several glands.

Following thyroidectomy in the fowl the large basophiles which are present in controls from 10 days on do not develop. The basophiles which do develop remain small. Large T cells, however, are found and appear within 10 days in chicks thyroidectomized at 30 days old (figs. 8, 9, 10, 11). These T cells do not arise in the region where basophiles normally develop in controls, but in the region of the peripheral embryonic cells (figs. 11 and 12). Since embryonic or chromophobic cells are the only cells found in this region, it is clear that T cells may develop directly from chromophobes. In fact all stages of transition are easily traced. The growth of the T cells seems rapid. At any rate in early and even later stages of growth the cytoplasm is sparsely granular and must contain a high percentage of water. The original reddish cytoplasm, often in fragments, sometimes scattered and sometimes segregated near the cell wall, can still be identified. The nuclear caps or metabolic centers formed by the Golgi substance,

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Figs. 1, 2, 4 and 5 Basophiles of controls showing prominent nucleoli.

Figs. 3 and 6 Large basophiles from castrates showing much enlarged nucleoli. In 3 the chromatin shows faintly.

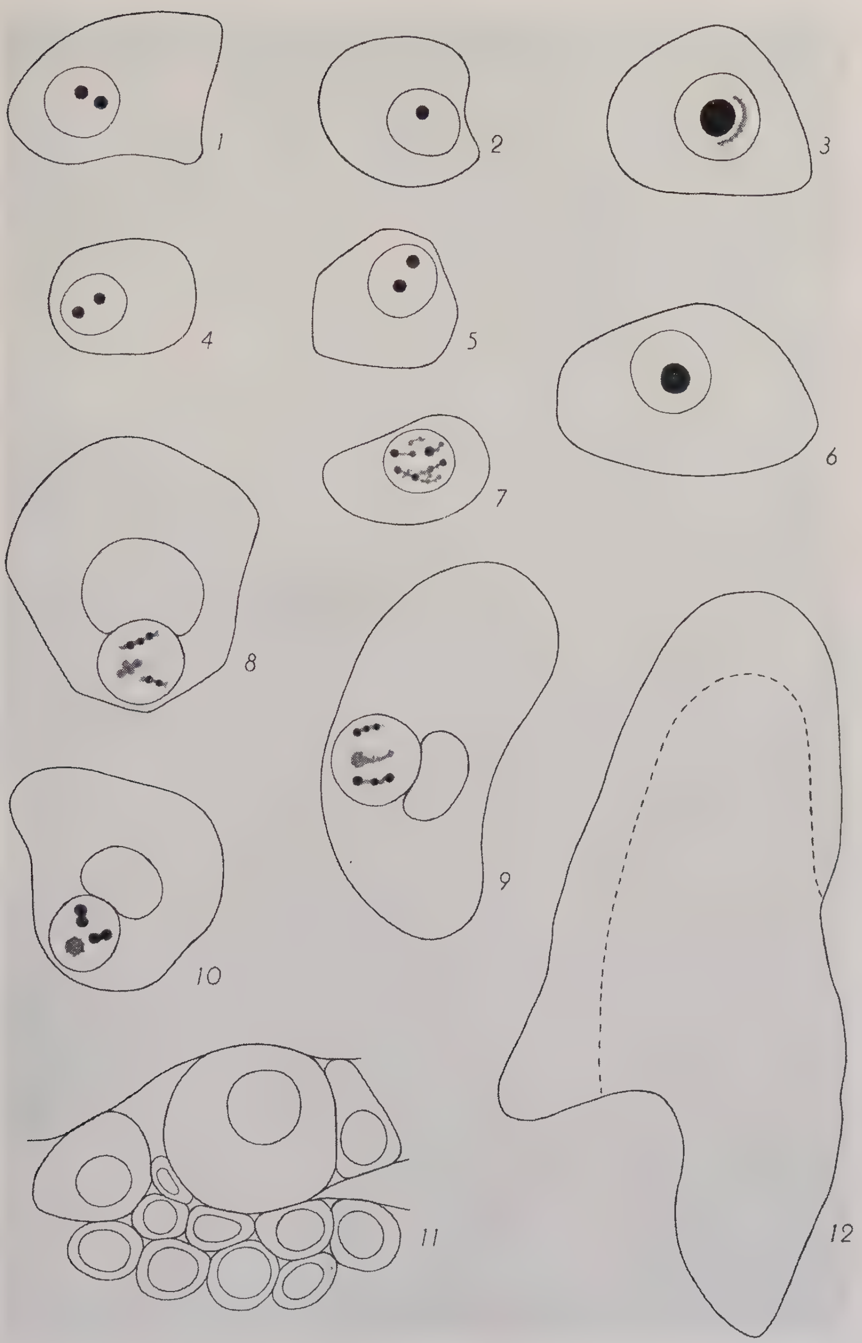
Fig. 7 An acidophile showing nuclear structure. The small nucleoli appear as knots in the chromatin network.

Figs. 8, 9 and 10 Three T cells. The nuclear structure is similar to that of the acidophile. Note the enlarged Golgi substance which is similar to that found in secreting basophiles and acidophiles.

Fig. 11 A sketch of several cells at the periphery of the pituitary of a thyroidectomized chick. The small cells are chromophobes, the larger cells are developing T cells.

Fig. 12 A median sagittal section through the anterior pituitary of a thyroidectomized chick. The T cells lie, for the most part, between the broken line and the periphery.

All figures with the exception of 12 have been drawn to the same scale.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Figures 1 to 12

mitochondria, and other unknown chemical substances, stand out prominently as dense greenish areas in the watery cytoplasm. These centers grow until eventually they are as large as the metabolic centers of large basophiles of controls and castrates, and the Golgi substance (figs. 8, 9 and 10) is practically identical with that found in well developed secretory basophiles. The cytoplasm gradually acquires a denser granular appearance and the tendency to stain with the fuchsin disappears. The cytoplasm, while different in the early stages, appears in the end like the cytoplasm of basophiles. There is no vacuolation, however, as in rats but there is little or no vacuolation in the basophiles of either controls or castrates in the fowl. While the cytoplasmic structure of the mature T cells is very similar in appearance and staining reaction to the cytoplasm of functional basophiles, the nuclear structure is more like that of the acidophiles. In the nuclei of basophiles the chromatin is either not visible or only faintly so, but there are one or two and occasionally three spherical nucleoli which gradually grow larger with the growth of the cells until finally they form conspicuous landmarks by which the basophiles can be identified (figs. 1, 2, 4 and 5).

In the nuclei of the acidophiles the chromatin is clearly visible and lies scattered about in flocculent strands (fig. 7). The nucleoli, if any are visible, are small and inconspicuous in comparison to those of the basophiles. The mature T cells, then, have cytoplasm similar to the cytoplasm of mature basophiles but with nuclei similar to those of acidophiles (figs. 8, 9 and 10). They look like functional secretory cells and there is no evidence in the fowl to the contrary. Are they modified basophiles secreting a gonadotropic hormone or are they enlargements of cells which in normal chicks secrete the thyrotropic hormone? The answer to this question may have to wait for more refined techniques and more accurate methods of measurement, but in the fowl the two cells are different in origin and structure. They do not arise in the same regions of the pituitary. They do not respond to the same stimuli. The basophiles multiply and enlarge following castration while T cells do not develop. T cells develop following thyroidectomy while B cells remain undeveloped.

Experimentally induced hyperthyroidism. Evans and Simpson ('30) reported that the pituitaries of female rats, fed 1 gm. fresh beef thyroid daily, had greater gonad stimulating effects than pituitaries of controls. Similar results were obtained by Van Horn ('31), as a result of feeding 125 to 200 mg. of desiccated thyroid daily for 6 weeks. Cohen ('35) extended these results to male rats. Severinghaus, Smelser and Clark ('34) described basophilic changes in the pituitaries of male rats following thyroxin injections or thyroid feeding and state that the basophiles increase in size and number and that these enlarged cells are similar to those found following castration and thyroidectomy. Jaap ('33), described testis enlargement and increased sperm formation in ducks fed on desiccated thyroid and found the increased size and activity varied with the dosage.

The pituitaries of several series of thyroxin injected chicks have been studied. The ages and dosages follow:

SERIES	DOSAGE	AGE KILLED
1	10 γ daily 5–29 days	30 days
2	20 γ daily per 50 gm. of body weight 10–28 days	29 days
3	5 γ daily per 100 gm. of body weight 10–39 days	40 days
4	10 γ daily per 100 gm. of body weight 10–39 days	41 days
5	20 γ daily 10–20 days; 50 γ daily 21–39 days	40 days
6	100 γ daily per 50 gm. body weight 10–16 days and 50 γ daily per 50 gm. body weight 17–40 days	41 days

For descriptive purposes the first five series may be grouped together. The body, comb and gonad weights approach those of controls and the pituitary picture is also similar to that of controls but the basophiles are less well developed. In series 6 the dosage may have been pathological. At any rate the body, comb and gonad weights were low and the pituitaries were small. The basophiles were undeveloped. In none of the series was there an enlargement of the basophiles and hence there were no changes comparable to castration changes. T cells did not form. The acidophiles were variable as in controls. In some pituitaries depletion of granules was evident.

Thyroxin injections following thyroidectomy. There is very little to report except that thyroxin injections tend to prevent thyroidectomy changes and to keep the pituitary similar to that of controls. There were no T cells.

Thyroidectomized capons. Authors generally agree that the basophiles increase in size and number and that the acidophiles recede following castration. Certainly this statement applies to fowls. In some species the enlarged basophiles become vacuolated but this is not true in fowls which I have studied. I shall use the term "castrate cell" to apply to the enlarged basophiles whether vacuolated or not. A study of thyroidectomized capons should throw some light on the similarities of or differences between castration cells and T cells. Specifically what are the effects of castration on T cell formation in thyroidectomized chicks and what are the effects of thyroidectomy on the formation of castrate cells. If castrate cells and T cells are similar, and if thyroidectomy is superimposed upon castration or vice versa we might expect an accelerated effect and Zwecker ('37) describes greater hypertrophy of the pituitary following thyroidectomy and gonadectomy than following either thyroidectomy or gonadectomy alone. If the two changes are different, however, they should exhibit divergent tendencies. I have already said that castrate cells and T cells are different as to place of origin and as to nuclear structure. My observations have been made upon 40-day-old chicks castrated between the fourth and sixth days and thyroidectomized either on the tenth or twentieth day; also upon 62-day-old chicks castrated on the fifth day and thyroidectomized on the thirty-fifth day. Speaking generally there are no capon effects when young chicks are castrated at 4 to 6 days and thyroidectomized at 20 days or younger. In White Leghorn control castrates enlarged basophiles were visible at about 20 days. In the 62-day-old chicks castrated at 5 days and thyroidectomized at 35 days, castrate effects were marked. Here, of course, castrate effects had developed before thyroidectomy. The evidence, which is not conclusive, indicates that the large basophiles of these 62-day chicks had begun to show regressive changes. Further studies are needed, however, on older chicks. T cells were found in both the 40- and 62-day age groups but they were only slightly developed in comparison to those found following thyroidectomy alone. In other words, the absence of the sex glands and presumably the absence of the sex hormone influences adversely the development of T cells. This effect on the T cells is the opposite of that produced on the basophiles by castration alone. There is a difference, however, in that the thyroid is present in one instance and not in the other.

In all cases where castration and thyroidectomy were performed early, body and comb weights were as low or slightly lower than following thyroidectomy alone. The pituitary size was much smaller (3021 to 3977) indicating clearly there was a retardation rather than an acceleration of the development of the basophiles.

Capons injected with thyroxin. As previously stated, several authors have concluded that an experimentally induced hyperthyroid condition in normal non-castrate animals stimulates the output of gonadotropic hormone and Severinghaus, Smelser and Clark ('34) described basophilic changes similar to those following castration. We have injected capons with thyroxin to further test the relationships, if any, between the castrate and T cells. My pituitary observations have been based upon four series of experimental chicks. In each series, castration was performed at 3 days of age and the chicks killed at 40 days. In series 1, the chicks were injected daily from the tenth to the thirty-ninth day with 5 γ thyroxin per 100 gm. body weight. In series 2, injections were doubled for the same period. In series 3, injections were 20 γ daily from the eighth to the twentieth days and 50 γ daily from the twenty-first to the thirty-ninth day. In series 4, the quantity of thyroxin injected was increased to 100 γ per 50 gm. body weight from the tenth to the sixteenth days and 50 γ per 100 gm. body weight from the seventeenth to the thirty-ninth day. The results of the first three experiments were very similar. There was no acceleration of castrate changes and there were no T cells. Instead, basophilic changes which follow castration alone were slightly retarded and pituitary size was less than in capons. The dosage in series 4 was much larger, perhaps even pathological. The result was a complete block of all castrate effects.

Capons injected with testosterone and thyroxin. It is well known that injections of androgens into castrate animals retard or prevent the usual pituitary changes which follow castration. In an experiment to test the effects of testosterone and thyroxin when injected simultaneously into capons, chicks were castrated at 3 days and injected with 10 γ thyroxin and 10 γ testosterone per 100 gm. of body weight from the tenth to the thirty-ninth days and killed on the fortieth day. The result was the block of all capons effects and the block was more effective than when capons were injected with 10 γ testosterone alone.

Dwarfs. Dwarfs are common among animals in general and may be due to many different causes. Following thyroidectomy in the fowl, body growth is retarded and, as stated previously, the anterior pituitary shows cytological changes, one of which is the development of T cells. The question naturally arose whether T cells are present in dwarfs. The answer is that T cells were found in the pituitaries of six of fifteen dwarf chicks studied. The birds in which many large T cells were present were 6 months old and were obtained from the Department of Poultry Husbandry of the University of Illinois through the courtesy of Dr. Nalbandov. Five were Rhode Island Reds from the same brood and one a White Leghorn. The White Leghorn and two of the Reds were females. The other three Reds were males.

The gonads in all were small, measuring about the same as those of normal White Leghorn chicks 3 to 5 weeks old. The thyroids were small and when sectioned contained very little colloid. The basophiles were undeveloped, indicating a low output of gonadotropic hormone. The anterior pituitaries of these six dwarfs were essentially the same as those found following thyroidectomy and the conclusion seems to be justified that the pituitary modifications were due to the undeveloped state of the thyroid.

In view of the fact that the acidophiles may secrete a growth hormone it is of some interest to find in these dwarf chicks that the acidophiles are well developed. This statement applied to all dwarfs studied.

Conclusions and discussions. The results of my observations in fowls may be stated as follows:

1. Body, comb and gonad weights remain low in chicks thyroidectomized when young.

2. Thyroidectomy cells or T cells form in the anterior pituitary of the fowl following thyroidectomy.

3. With respect to place of origin and nuclear structure the T cells are different from the large basophiles of both normal and castrate birds.

4. The T cells appear, from all points of view, to be secretory cells. There is no vacuolation and no indication of degenerative changes.

5. T cells originate for the most part, if not in all cases, from chromophobes.

6. In all thyroidectomzied chicks, including thyroidectomized capons, the pituitary is smaller than in controls.

7. Following thyroidectomy there is a retardation rather than an acceleration of basophilic development.

8. No castrate changes occur and only a few small T cells develop in 40-day-old chicks caponized when 3 to 6 days old and thyroidectomized prior to 20 days.

9. In 62-day-old chicks caponized on the fifth day and thyroidectomized on the thirty-fifth day, castrate changes are marked and T cells are present in small numbers. Here castrate cells and T cells are present in the same individuals and are morphologically distinct.

10. T cells do not develop following injections of either small or large doses of thyroxin in normal, thyroidectomized, or caponized chicks.

11. There are no castrate effects following thyroxin injections in normal chicks. The basophiles are similar to those in controls following small doses but remain small following larger doses. The acidophiles are prominent and degranulating.

12. Thyroxin injections following thyroidectomy prevent thyroidectomy changes.

13. Injections of small doses of thyroxin into capons cause little if any change; larger doses retard the usual castrate changes.

14. Following injections of both thyroxin and testosterone in capons, castrate changes are blocked more effectively than when either thyroxin or testosterone is injected alone.

15. In dwarf chicks with undeveloped thyroids the pituitary is similar to the pituitary following thyroidectomy. T cells are present, the basophiles undeveloped and the acidophiles prominent.

The conclusions stated are obviously at variance in many respects with the conclusions of most authors whose studies have been based on the rat and other mammals. Are these differences reconcilable or are they expressions of fundamental differences between species? All authors seem to agree that thyroidectomy cells arise following thyroidectomy and the fowl is no exception. There is not agreement, however, with respect to what these cells are cytologically or functionally. Are they modified basophiles as most authors seem to think, or do they arise directly from chromophobes as described by Bryant? Are they secretory cells or are the changes degenerative in character as Severinghaus and Guyer thought them to be? If the cells are secretory do they secrete a gonadotropic hormone or the thyrotropic hormone as the work of Zwecker indicated?

From the points of view of origin, location and morphology, the T cells of fowls are unlike castrate cells and the large basophiles of controls. In most cases, if not all, they arise from chromophobes. They are different from any other type of cell in the pituitary and the conclusion is inescapable that they are not modified basophiles. There are other reasons for believing that the T cells and castrate cells in the fowl are different. Thyroidectomy retards basophilic development and this applies to capons where, following castration alone, basophiles become greatly increased in size and number. In fact, there are no castrate effects in capons if thyroidectomy is performed prior to 20 days of age. If castrate and thyroidectomy effects are identical, however, a summation of the two effects might be expected. In capons thyroidectomized after castrate cells have formed, T cells and castrate cells may be found in the same pituitary. Logically it does not seem reasonable that castration and thyroidectomy effects on the pituitary should be identical. Neither does it seem logical that fundamental differences following thyroidectomy should be so diverse as those described in rats and fowls. If T cells in the rat are modified basophiles similar to castrate cells, then they should be modified basophiles in the fowl. The evidence in the fowl, however, is contrary to such a conclusion. My suggestion is that the cellular changes following thyroidectomy in the rat are so nearly like those following castration that morphological differences are not easily detected while in the fowl the differences are greater and more readily observed.

As to the function of the T cells the answer is still more confused. Perhaps they have no function but the morphological evidence in the fowl indicates they are secretory. If they are secretory do they secrete a gonadotropic or the thyrotropic hormone? Assay tests on the rat are so variable that no conclusion is possible and as yet we have completed no such tests in the fowl. From cytological studies alone, however, the T cells in the fowl are not basophiles and if they are not basophiles they probably do not secrete a gonadotropic hormone.

There is a certain analogy between T cell formation following thyroidectomy and castrate cell formation following castration. The large castrate cells of capons arise, according to our present interpretation, because, following castration, the modifying influence of the sex hormone on the basophiles is removed. A similar interpretation may be applied to the T cells. In normal fowls the thyroid secretions may provide a check on the development of T cells which is removed with the removal of the thyroid. The conclusion seems fairly well established that castrate pituitaries contain a larger quantity of gonadotropic hormone than pituitaries from controls. It might be logical to expect the T cells to secrete the thyrotropic hormone and results of assay tests in the chick will be watched with interest.

Such a suggestion is contrary to the conclusions of Marine, Rosen and Spark ('35) and Severinghaus ('37) that the acidophiles secrete the thyrotropic hormone but their evidence is far from conclusive.

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BEHAVIOR OF LYMPHATIC VESSELS IN THE LIVING ~~R~~AT

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FIVE FIGURES

The mechanics of the propulsion of lymph in the mammal have remained a problem since the discovery of a lymphatic circulation. That lymph moves within its system of vessels is an accepted fact, confirmed many times a year in the classical laboratory experiment where the lymph from the cannulated thoracic duct is collected under various conditions. Explanations of the forces involved in this forward propulsion vary in detail but agree upon the principle that the lymphatic system itself plays a passive role. This obstinate aversion to giving serious consideration to the active participation of the lymphatic vessels is indeed surprising in view of the considerable body of evidence to the contrary.

The possibility that the walls of lymphatics might assist in the propulsion of lymph was indicated by Sheldon, 1784. After dissecting cervical lymphatics in the necks of living dogs, he noticed that upon mechanical stimulation the vessels contracted. His conclusion, "— that the muscular power of the vessel, assisted by the collateral pressure produced by respiration, and by the adjacent muscles and arteries will impel the fluid towards the heart," has remained appropriate since the time his work was published. A similar view was held by Hewson in his works published by Gulliver, 1846. In summarizing the action of lymphatics, Hewson states that those vessels which convey chyle are believed to have muscular fibers which, stimulated by fluid, may contract peristaltically and press the fluid forward from one pair of valves to another. He mentions that the accessory force of pulsating arteries, "inconsiderable as it is, may promote the passage of lymph."

Heller, 1869, was the first, actually, to describe rhythmic contractions of lymphatics. He found a lymph pulse of 10 per minute in the lacteals or mesenteric lymphatics of the guinea pig. This rhythm was entirely different from the respiratory rate of 40 and an arterial pulse of 120. His observations received little attention. Again in 1910, a rhythmic pulse of 15–18 per minute in the lacteals of the rat was described by Lieben. He added the observation that the rate of contraction could be altered by dropping certain drugs on the vessels. His statement of a similar condition in the mouse has been challenged by later workers. The discussion was concluded with the assertion that contents of lymphatics are advanced by muscular contractions and by activity of the gut against the radix mesentery.

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In a second article Heller ('11) chided Lieben for failing to read his paper of 1869 on the subject of rhythmic contraction of lymphatics. Unaware of this remonstrance, Carrier ('26) "discovered" pulsating lymphatics again. She saw these thin-walled vessels, richly supplied with valves, in the wing of the bat. Their contraction occurred at the rate of 12–15 times per minute.

The following year rhythmical contractions of the lacteals of the rat and guinea pig were redescribed by Florey ('27a). This report agreed essentially with that of the previous investigators. In a second paper ('27b) Florey stated that the lacteals of the cat, dog, rabbit, squirrel, hedgehog, mouse, guinea pig, and man do not exhibit similar rhythmic contractility. In their study of the histology of the lymphatics in various animals, Carelton and Florey ('27) were unable to explain why the lacteals of rats and guinea pigs are contractile and those of other mammals under the same conditions are not. The absence of rhythmic contractions in the larger lymphatics of the rabbit was observed by Clark and Clark ('32) to apply also to the peripheral system, for they describe the movement of lymph in the ear as a bobbing back and forth, synchronous with the heart beat or respiration, and resulting only in a sluggish, jerky, forward progression.

In their study of lymphatic capillaries of the mouse ear, Hudack and McMaster ('32) did not see active contraction of these vessels. In a later paper ('33) they commented on the rapidity of the movement of lymph in the lymphatics draining the skin of the human forearm.

During 1933, while the paper describing the contractions of the mesenteric lymphatics was in press, one of us (Webb) encountered Florey's work on the same subject. His observations agreed with those of Heller, Lieben, and Florey, but he had been fortunate in obtaining photographs of different phases of their contractions. Later ('34) a complete record was recorded on micro--cinematographs, a copy of which was placed in the Film Depository of The Wistar Institute and reviewed recently by Clark ('41).

Pullinger and Florey ('35) report pulsations of the thoracic duct in guinea pigs, and rhythmic contractions in the lymphatics of the thigh in both the rat and guinea pig. No contractions were observed in the lymphatic capillaries of the mouse's ear. Pfuhl and Wiegand ('40) were so impressed by the arrangement and amount of muscle in the region of the lymphatic valves in the guinea pig's omentum that they called these areas "microlymph hearts." It is their belief that these lymph hearts are active in propelling lymph forwards.

Several investigators, who have used the dog as the principal subject for their experiments, find little evidence for ascribing an intrinsic function to the lymphatic vessels in the movement of the lymph. During periods of complete physical inactivity in these animals Weech, Goettsch and Reeves ('34) noted that lymph flow in the peripheral lymphatics ceases entirely. Capillary filtrate accumulates in the interstitial spaces and enters the lymph channels at once when activity stimulates the "pumping action of the valves." Yoffey and Drinker ('38) found that massage was usually necessary to induce the flow of lymph from the nose to the cervical lymph nodes in the dog. This was verified by McCarrell ('39) who extended the work by determining the pressure necessary for flow in the cervical lymphatics. However, spontaneous flow of lymph in the cervical lymphatics of the monkey was reported by Yoffey and Drinker. In a rather sweeping generalization, Drinker ('39) lists good circulation and active peristalsis as the factors which cause free movement of lymph from the intestine.

In the present investigation on bats, we have endeavored to analyze the movements not only of the mesenteric and wing lymphatics but also those of the body wall. The bat was chosen because of the ease with which these vessels can be studied in the intact wing. Thus, we have been able to compare the action of lymphatics which have been exposed by operative procedure with those of normal vessels.

MATERIALS AND METHODS

The common Brown or Cave bat, Myotis lucifugus, and its close relative the Say bat, Myotis subulatus (Hahn, '09) have proved to be best suited for the studies here reported. Their intermediate size and the texture of their wing membranes make them ideal for studies of the cutaneous lymphatics. They also give satisfactory results for routine studies of the mesenteric lymphatics. In certain cases, the larger species, Myotis vilefer, because of its sturdier structure and wider mesentery, is more suitable for studies of the lacteals. The small Georgian bat, Pipistrellus subflavis, although showing similar lymphatic characteristics is too small for routine use.

These bats may be collected in large numbers from almost any cave in southern Indiana from October through May. During the remaining months they leave the caves and then only an occasional specimen is obtainable. During the winter months it has been easier to collect the bats as needed from the caves rather than to attempt keeping large numbers in the laboratory. Small numbers may be kept in the refrigerator at approximately 5°C. for several weeks. They should be placed in pans, covered with mesh wire netting from which the bats hang over a centimeter of water. Large clusters should be avoided as their own heat production in such groups prevents them from going into hibernation. An hour before use they should be taken from the refrigerator and placed in a suitable cage at room temperature.

While it is possible to mount and study the wing lymphatics without any anesthesia, such preparations offer only limited use. Direct comparison has shown that no important change in lymphatic behavior is introduced by light anesthesia. Of several anesthetics tried, Sodium Amytal (Lilly) has been the most satisfactory. Dosages of approximately 0.075 mg. per gram of body weight, given intraperitoneally in 0.25 cc. of water have proven adequate.

In the wing any portion of the membrane may be used, but a rough standardization was attempted by restricting the field to the lymphatics that accompany the main vascular bed in the membrane between the fifth finger and body wall. The bat was mounted, usually on its back, on a cork slab in the center of which was embedded a large glass slide. The area for study was spread over the glass portion and held in place under slight tension by pins placed peripherally. Contact between the under surface of the wing and the glass was made with a film of liquid petrolatum.

Visual observations of the lymphatics with all powers of the compound microscope may be made with such preparations. If the upper surface of the membrane is covered with petrolatum an hour or two before use, partial clearing of the epidermis occurs without injury to the intradermal structures. This aids materially the clarity of direct visual observation but it is impossible to obtain successful photomicrographs with such technique. The difficulty lies in the scattering of the light by the cells of the upper epidermis and the resultant formation of blurred images of the lymphatics. Nevertheless, these normal, untreated preparations are useful in the analysis of the lymphatic action which is seen here in its most vigorous manner. They have the disadvantage of not permitting the permanent recording of their activity.

For photographic records and experimental procedures the upper epidermis is removed. A slight incision is made in the epidermis which is then stripped back with fine forceps. The loose nature of the connective tissue allows this to be done with a minimum amount of damage to the haemal and lymphatic systems. The exposed surface should be kept small in area but several such windows along the lymphatic system may be prepared. The field is bathed with balanced salt solution and covered with a thin cover glass. In long periods of observation it is necessary to remove the cover glass occasionally and replenish the salt solution.

The lymphatics in the skin of the body wall were studied in exposed flaps. The bat is shaved and the skin reflected from an incision along the axillary line of one side. Either the back or abdominal areas may be used, although the latter is more successful due to the absence of a well-developed muscle layer. The outer edge of such a skin flap will be bloodless but no interference with the blood or lymph circulation in the inner portion is evident. The flap is pinned out under slight tension over the glass portion of a suitable slab, and the exposed surface covered with salt solution and protected by a cover slip. Such preparations have remained in excellent condition for several hours.

To observe the mesenteric lymphatics and their large lymph node, the bat is mounted on its side and the entire intestine carefully extruded through a median abdominal incision into a pool of salt solution. Despite hemorrhage, satisfactory results are obtained by pinning the intestine to a semicircular cork ring fixed on the glass slide at a suitable distance from the bat. The pins pierce the free border of the intestine opposite the larger blood channels. The intestine with its mesentery is immersed in salt solution and the whole field protected by a cover slip.

The hemorrhage that usually develops after a short interval at the site of the pins is the result of intestinal activity, and limits the life of such preparations. The extravasated red corpuscles appear soon in great numbers in the mesenteric lymphatics and are carried rapidly to the large node. The mechanism of their entrance into the lacteals offers an intriguing problem for further study which might well shed light on the mode of entrance into these vessels of absorbed fat droplets in the normal animal.

All types of rhythmical activity of the lymphatics described in the text have been recorded as cinematographs on Eastman Super-XX, panchromatic, 16-mm. film. The Spencer equipment for motion photomicrography was used to adapt the 16-mm. motion picture camera to the microscope. This equipment is set up with the focusing side tube and light-splitting prism attached to the revolving nosepiece of the miscroscope. The prism divides the light, sending 90% to the camera and 10% to the eye. A focusing telescope fits over the regular microscopic eye piece. It contains a rectangular diaphragm and can be revolved to show the exact area being photographed. The Bell and Howell 70-DA Camera is equipped with a Critical Focuser. Such a device permits the image of the subject studied to be viewed on a ground glass which is in the same plane as the film. By this means, a final check may be made on the sharpness of focus before the film is exposed.

The prepared animal is placed on the stage of the microscope and the frame of the mounting slide is engaged in the mechanical stage. Thus, it is possible to study the living animal in the same manner as one studies tissue mounted on a slide. The source of light is from the ordinary microscope lamp and is transmitted through the thin tissue of the living animal. During the operation of the camera, the field is under observation. The resulting records obtained in this manner are incorporated in a film which will be available to those who are interested in the subject.

The photographs used as illustrations in this article are enlargements of individual frames from the 16-mm. film. The technical difficulties encountered in making such enlargements are almost insuperable. This fact should be kept in mind when one makes a critical appraisal of the figures. Of the photographs illustrating the movement of the living lymphatics, only the group in figure 2 has been retouched to accentuate the outlines of the vessels.

OBSERVATIONS

Before describing the activity of the lymphatic vessels, it is necessary to mention briefly some characteristics of their anatomical structure. In far too many cases both anatomical and physiological studies of the lymphatics have been confined by technical reasons to the larger vessels. Fortunately, the wing membrane of the living bat offers an exceptional opportunity for microscopic study of the lymphatic radicles.

The accepted idea of the lymphatic capillaries in the skin of mammals is a plexus of irregularly-shaped anastomosing vessels 30 to 60 microns in diameter. This plexus is connected to the main lymphatic channels by irregularly-spaced collecting vessels that are richly supplied with bicuspid valves. The presence of such valves in the collecting and transporting lymphatics insures the centripetal flow of lymph. Their absence in the peripheral capillary plexus implies that any substance which should chance to enter it, such as bacteria, could spread throughout this system with considerable ease.

Analysis of the form and distribution of the lymphatics in the wing membrane of a live bat shows that these ideas should be modified, at any rate, for this animal. Figure 1 is a line drawing made to exact scale of a typical region in the wing membrane, which presents the complete lymphatic distribution in the area. Only a fraction of the blood capillaries were included to avoid confusion. The inclusion of all of the lymphatics in the territory is possible because the membrane is so thin that only one layer of lymphatics is present. The capillary tributaries, which are discussed in detail later, drain to a system of collecting vessels and serve the membrane in their vicinity. There is not the double plexus of superficial vessels which one sees in the rabbit ear (Henry, '33).

The vessels of the lymphatic system in the wing area may be described under three main subdivisions although, as is usually the case, intermediate types are seen. Those which accompany the larger blood vessels may be called transporting lymphatics (fig. 1, t. l). Their average diameter is usually the same as the artery they accompany, but this seems to depend in part on the state of tone of their walls. The same vessel may appear twice its former width along its entire length when observed at other times. Usually the tone appears to be low and the normal width of the quiescent segment is near its physiological maximum. The contour of their walls is irregular and constantly changes in appearance because of localized contraction at numerous points. They are supplied with valves, not only at the junction of all branches, but also along the course of the main channel (fig. 2). Typical bulb-shaped sinuses are found surrounding the valve-flaps and appear to be the place of origin of the segmental type of contraction characteristic of the transporting channels. The segment may be defined as that portion of the vessel extending between the bases of two adjacent valves. Analyses of the action of these segments, made from a study of consecutive frames of a motion picture, reveals the segmental nature of the contraction and does not support the visual impression that it is peristaltic in form. The unit which includes the sinus and, as a rule, most of the vessel to the next valve downstream is involved in a single simultaneous contraction. Localized contractions along the vessel wall and the mechanical distortion of any given segment due to the action of adjacent segments make detailed analysis extremely difficult.

As the transporting lymphatics are followed peripherally, that is, upstream, their segmental character gives way to a different structural

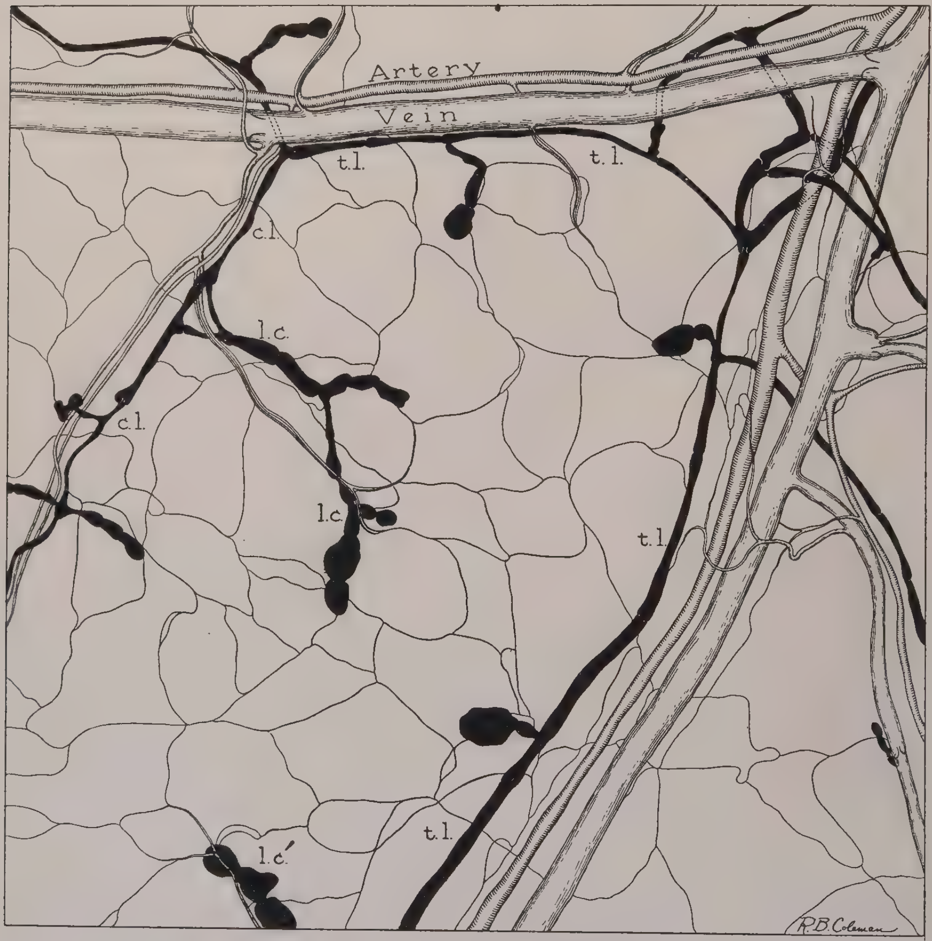

Fig. 1 Lymphatic and blood vessels in area of bat's wing at a bifurcation of two large vascular channels, × 10. Lymphatics black, blood vessels shaded, and blood capillaries represented as lines. Lymphatic capillaries, l.c., begin as spheroidal sacs, connected to collecting lymphatics by a series of oval-shaped bulbs. l.c.', bulb estimated to have dimensions 720 by 450 microns. As capillary approaches larger lymphatic, connections between bulbs have more valvular appearance. Collecting lymphatics, c.l., receive lymph from bulbous capillaries, accompany small blood vessels and are definitely valved. Transporting lymphatic, t.l., associated with larger blood vessels. Valves segmentally arranged. Rhythmic activity is greatest in bulbous lymphatic capillaries, is confined to region of valve in collecting lymphatics, and is segmental in transporting lymphatics.

pattern. The sinus portion at the valve becomes larger and more prominent while the remainder of the segment is modified into an irregular thin-walled connecting passage. When seen in its purest form, such a vessel originiates at the junction of one or more capillaries and joins the larger transporting lymphatics. Because of its relationship to the capillaries the term collecting lymphatic (fig. 1, c.l.) is used to designate it. Vessels of this kind usually accompany the venules — small veins — to the larger trunks. As the collecting branches from a given region unite to form larger vessels, a retrograde flow of lymph is prevented by well-developed valves.

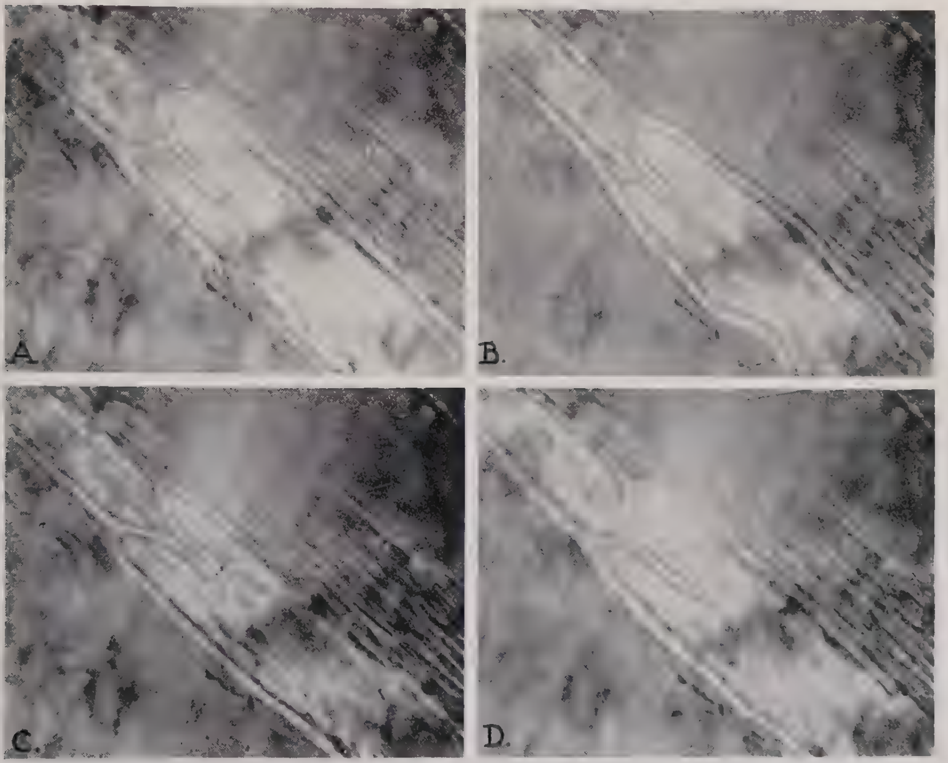

Fig. 2 Enlargements of microcinematographs of a series of changes occurring in the walls of a transporting lymphatic in a bat's wing during a segmental contraction, × 160. Position of valve indicates that flow of lymph is from left to right. (A) Portion of segment to the left of base of valve relaxed. Sinus and part of segment to right of valve moderately relaxed. (B) Left segment contracted, valve sinus contracting, and right segment still relaxed. (C) Little change in left segment. Valve sinus and right segment contracted. (D) Both portions of the segments and the valve sinus have returned to a state of rest. These photographs have been slightly retouched.

Some collecting vessels are found that extend between two transporting channels. However, a study of the direction of the valve flaps along the course of such an interconnection shows an arrangement which prevents any mixing of lymph by this route. Somewhere along its course a stretch is found where sinus-like enlargements occur that possess no valve flaps. When flaps reappear at the farther end of the segment, they point in a direction opposite to those previously encountered. This mechanism allows lymph that enters the interconnection from a tributary capillary to pass towards either of the transporting channels, but prevents its passage from one to the other. Thus, despite appearances on first examination, no physiological anastomosis of collecting vessels may be said to exist.

The collecting lymphatic is characterized by the differentiation of its valvular regions. The sinus is enlarged, and its walls show a heavy concentration of cells that give a more substantial appearance to the whole structure. Usually they occur at the union of two or more branches, but occasionally they are seen also along the recipient vessel. The sinuses into which the valve flaps protrude are noticeable because of their relatively large size. Beyond this enlargement the vessel narrows rapidly and loses the extra cells within its walls. A further variation of this structural plan is found in the peripheral parts of the collecting system, especially in the short stems of the capillaries. Because of the absence of flaps, the valvular function is lost, and only the sinus enlargement with its cellular concentration remain.

These sinuses, whether valvular or not, possess a marked capacity for independent contraction. Their activity may be so powerful that the lumen of the sinus is obliterated although the rest of the vessel shows only mild irregular twitchings along its walls. Fine strands of connective tissue anchor the lymphatics to the surrounding structures and, as a result, the latter are displaced toward the lymphatics at each contraction. The swaying movement of the neighboring blood vessels, reflecting the active contraction of the valvular structures, often is very noticeable. Probably, it was this action which Carrier interpreted as a direct effect of the lymphatics on the vascular system. The structural form and powerful contraction of these modified sinuses give credence to the term "lymph heart," as applied by Pfuhl and Wiegand to the valvular sinuses in omental lymphatics of the guinea pig.

The lymphatic capillaries (fig. 1, l. c.) of the bat's skin show a unique development. The twigs of the collecting lymphatics begin as immense bulbar enlargements. Their walls are so thin they cannot be detected as definite structures except by their marked positive contractions. The number and size of these bulbous structures exhibit great variation. Either a single or a series of bulbs connected directly together may comprise the "capillary." In many cases branching occurs within a given chain of bulbs, and the area served is quite extensive.

These large bulbous divisions of the lymph capillaries vary in shape from the nearly spherical forms of terminal bulbs to more oval types when in chains. The dimensions of a bulb (fig. 1, l.c.′) as measured by an optical micrometer were 720 by 450 microns in the relaxed state. The diameter is considerably greater than that of the collecting or transporting lymphatics and exceeds vastly that of the blood capillary.

The rhythmical contraction of these capillary bulbs is more pronounced than that of any other channel of the lymphatic system. The sequence of the contraction in a chain is such that a centripetal movement of lymph is produced. Like the valvular enlargements of the collecting channels, they are anchored by connective tissue strands to the neighboring tissues. These strands may radiate outward in all directions and finally terminate at a considerable distance from the bulbous capillary. The deflection of the adjacent tissue resulting from each contraction gives rise to the appearance of vast moving areas when an entire chain is under observation. This activity permits their detection under the low power of the microscope when the outlines of the bulbous capillaries themselves cannot be observed.

The function of the rhythmical contractions of these bulbous capillaries is not altogether clear. With each contraction their volume estimated from the measured change in diameter, is reduced to approximately half of that in their quiescent state. It is doubtful that this quantity of lymph actually is discharged into the collecting vessels at the time. The small size of the capillary stem and the rate of flow within the collecting vessels do not support such a voluminous lymph stream. More direct evidence is available on this point. Occasionally, a white cell may be seen within the lumen of a valvular sinus where the stem of a large capillary joins the collecting system. Its forward progression with each wave of contraction along the bulbous capillary indicates that only a small quantity of lymph actually enters the collecting system. It is possible that some of the activity of these bulbous capillaries is associated with a mechanism for the passage of tissue fluid into the capillary system.

The activity of the lymphatic vessels, whether observed in capillaries, collecting or transporting channels, is a positive independent contraction of their walls. The rate of the rhythmical contraction varies and depends on many factors. There is no relationship between their rate of contraction and other rhythmical activity, such as respiration, heart beat, or the independent contractions exhibited by the veins in the wing membrane. The flow of lymph in any given vessel is intermittent, and the rate of the lymphatic pulse in converging vessels may be quite different. This is demonstrated strikingly in the following experiment.

In a preparation of the wing two windows were made, one at the site of junction of two large vessels and a second near the peripheral end of one of these branches. Blood and lymph circulations were excellent in both fields. The central field was protected with a cover glass, and the point of union of the two major transporting channels was brought into focus under the microscope. The peripheral field was covered with 2% trypan blue in balanced salt solution. Within a few minutes the dye had entered the lymphatics of the peripheral field and could be seen passing rapidly in a centripetal direction along the transporting vessel from that area. At the junction of this channel with the other branch in the central area, an interesting phenomenon was seen. There was a rhythmical alternation of colored and clear lymph in the lymphatic centrad to the union. This showed that the flow of lymph from either contributing transporting vessel was intermitent and that the activity in each channel was independent and often completely out of phase with the other branch. It is impossible to see how any action other than the independent contraction of the contributing vessels could bring about this result. Furthermore, the phenomenon repeated itself at every union of a major side branch with the dye-filled lymphatics.

McMaster ('42) reports a similar observation in his work on the lymphatics of the mouse ear. The point is not discussed except as this action is used to demonstrate lymph flow from quiescent regions. Later in his work, however, evidence is presented in support of the idea that lymph movement in such cases is dependent on the rhythmical pressure changes in the blood vessels. It is difficult to understand how a uniform variation of that type could produce alternating intermittent lymph flow from two adjacent regions. In the present work, the explanation becomes obvious since direct observation showed that the rhythmical pulsations of the vessels were out of phase.

The morphological types and the physiological activity described are not peculiar to the lymphatics of the wing membrane. Similar structural groups of lymph channels having identical activity are seen in the skin of the body proper. The existence of the same positive contractions in the lymphatics of this region emphasizes the generality of the action. The corresponding veins of the body do not show the independent contractility of their counterparts in the wing membrane.

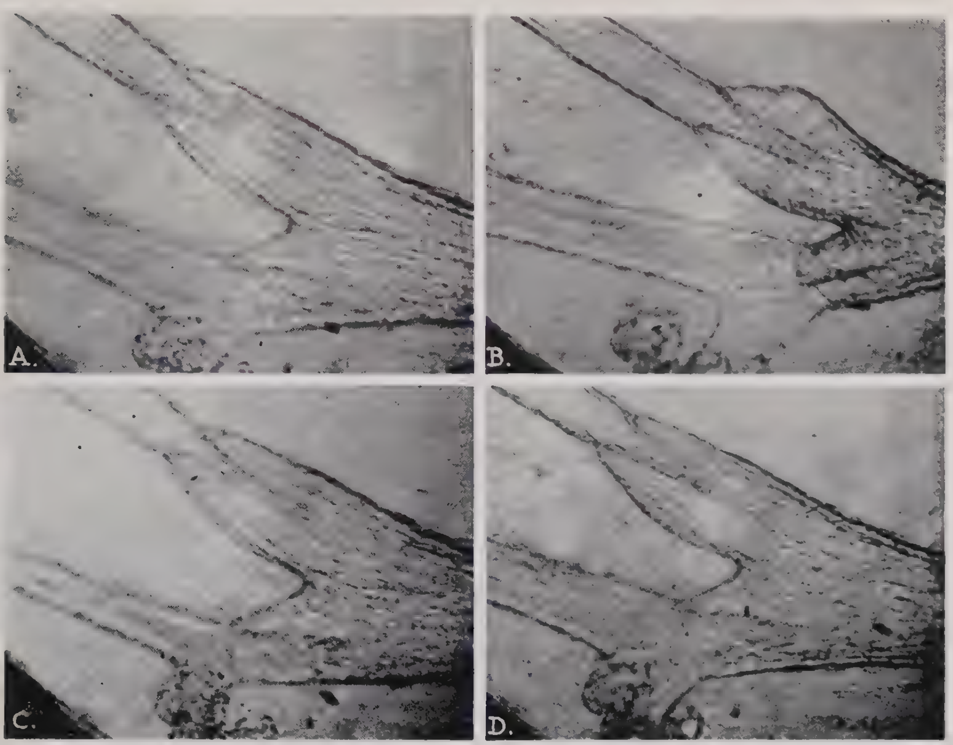

Fig. 3 Enlargements of microcinematographs of a series of changes occurring in the walls of three mesenteric lymphatics as they join to form a transporting vessel, × 160 (A) Tributary lymphatics in various stages of contraction. Main transporting lymphatic relaxed. (B) Tributaries in stages of relaxation, transporting lymphatic contracted. The flaps of the two valves of the larger vessel are approximated, preventing a retrograde flow of lymph. (C) Alternation in phases of contraction to that shown in B. Upper valve of main channel more relaxed than lower. (D) All vessels relaxed. The shift in position of the vessels in the four photographs is produced by the vigorous contractions of the various components as each pulls the group towards itself during contraction.

Yet there are some morphological differences between the lymphatics of the wing and of the body wall. They are: (1) transporting channels do not accompany the blood vessels; (2) collecting lymphatics are more numerous; (3) the valvular modification of "lymph hearts" are less developed; and (4) the bulbous capillaries are smaller and possess more definite walls. It may be noted again that no physiological anastomoses of the collecting vessels occur.

Further indication that the lymphatics of the bat conform to a basic type of structure and function is found in the mesenteric vessel. Here they show a rigid adherence to the segmental characteristics of the transporting vessels in both structure and activity (fig. 3). In this respect, they are more constant than simliar vessels of the rat whose general character they closely resemble. In the smooth contour of their walls, in the absence of contractions involving less than an entire segment, and in the marked mechanical displacement of one segment by the contraction of neighboring ones, they exhibit minor differences from the rat lacteals. The mesenteric lymphatics may be followed to their entrance into the large mesenteric node where all the transporting channels discharge their contents into the periphral sinus (fig. 4). Although the last segment of each contributing vessel showed rhythmical activity, the outer tunic of the node appeared to remain quiescent.

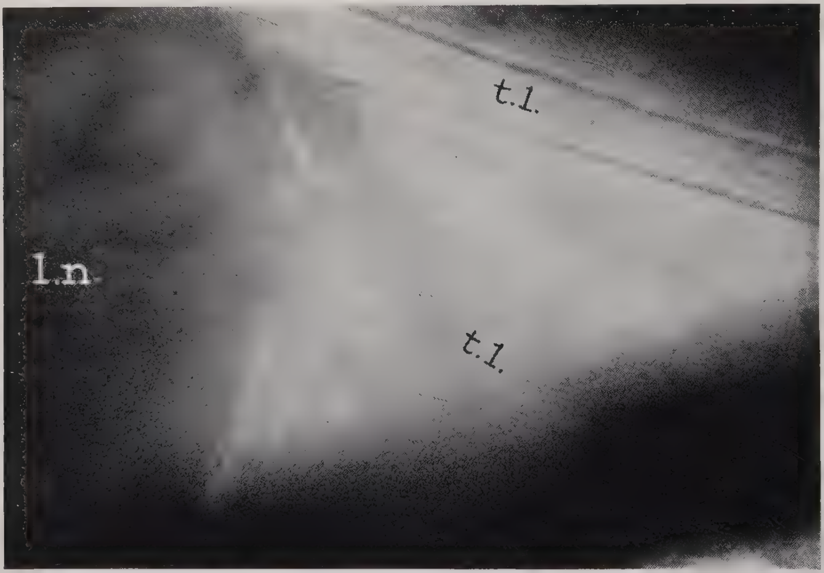

Fig. 4 Enlargement of a microcinematograph of a mesenteric lymph node, l.n., receiving two transporting lymphatics, t.l. × 160. The upper lymphatic, in sharp focus, pours its lymph into the peripheral sinus of the node. Its rate of contraction is different from that of the lower vessel, slightly out of focus. Each vessel contains a valve near its juncture with the peripheral sinus of the node.

Because of the absence of lymphatic capillaries in the mesentery of most animals, it is assumed that the lymph of its tissue enters the main channels or lacteals directly. In the bat mesentery, vessels that correspond to capillary enlargements and to collecting lymphatics may be found (fig. 5). They are not numerous and in those examples, so far seen, the usual activity was absent.[2] This should not be taken as definite evidence that under normal conditions they do not exhibit active contraction. For visualization it was necessary to place the mesentery under tension which may have interfered with the activity of the delicate structure. The outlines of the capillary enlargement were not visible except after staining with trypan blue, which appeared to fill the lumen rather than stain its walls.

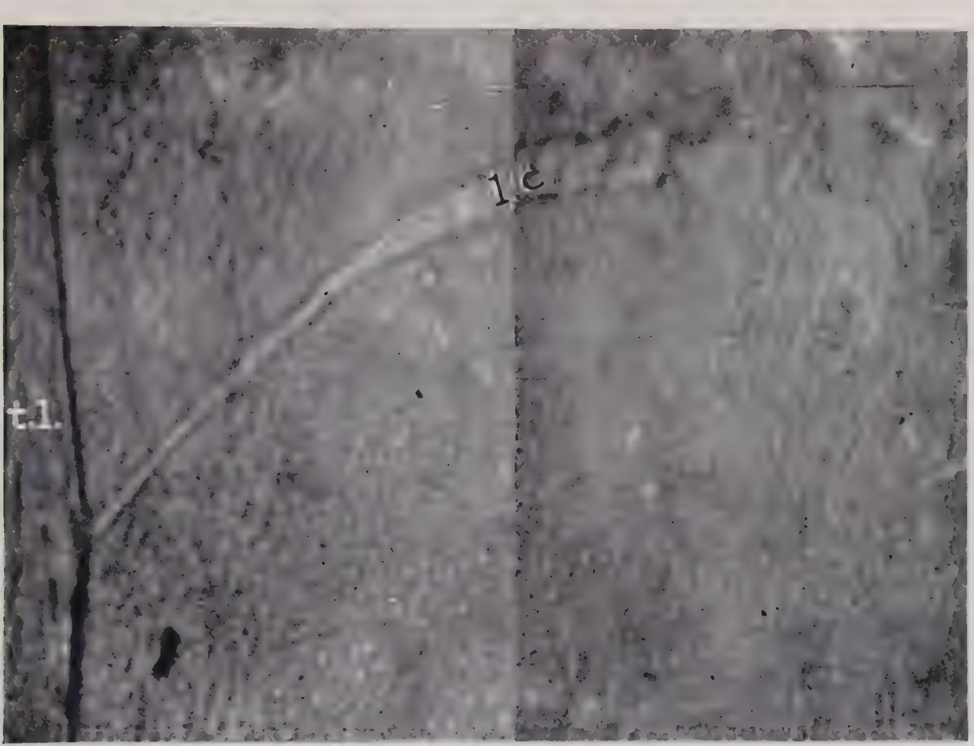

Fig. 5 A composite of two microcinematographs showing the complete extent of a mesenteric lymphatic capillary, l.c., from its origin to its junction with the lacteal, t.l., × 160. The capillary begins as a vague saccular enlargement. The walls become more definite as it approaches the larger vessel to the left.

SUMMARY AND CONCLUSIONS

The thinness of the wing membrane of the bat permits direct microscopical observation of all divisions of the lymphatics including the terminal twigs. Successful records of this activity under oil immersion objectives may be made with microcinematography by removing little more than the epidermis from one side. The activity of lymphatics in fields so prepared appears unchanged when compared with adjacent intact areas.

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[2] Later studies have shown that the bulbous capillaries of the mesentery are as active as those of the wing.

Three divisions of the lymphatic channels are recognized. In the larger transporting vessels the structure and activity are organized on a segmental basis. Each segment includes the entire lymphatic between two valves. The mesenteric lymph vessels are the best examples of this group.

The second pattern of vessels gradually evolves from the transporting channels towards the peripheral area. The segmental structure of the transporting channels is replaced by a thick-walled, expanded sinus representing a type of "lymph heart," while the remaining portion serves as a thin-walled collecting vessel.

The third division is represented by large bulbous capillary structures. Their walls are so thin that they are indistinguishable from the surrounding connective tissue except for their remarkable positive contractions. They begin as large spheroidal sacs which may be connected to the collecting lymphatics by one or a series of oval, bulbous structures. The rate of contraction is rapid, and their sequence propels the lymph centrally.

Physiological isolation of the peripheral lymphatics from each other is due to the form and location of their bicuspid valves which prevent retrograde flow.

The lymphatic system of the bat is characterized by its marked intrinsic activity. The nature of the activity differs with the type of vessel studied. The rate of rhythmic contraction of the bulbous capillaries is the most rapid, and the entire bulb participates in the movement. The ryhthmic activity of the collecting vessels is confined chiefly to the valvular areas. In the transporting lymphatics the movement is segmental. Each segment is confined to the region between two adjacent valves. The rhythmical contraction of the lymphatics is universal in the bat, for they may be observed in the wing, in the body wall, and in the mesenteries.

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BEHAVIOR OF ULTIMOBRANCHIAL TISSUE IN THE POSTNATAL THYROID GLAND: THE ORIGIN OF THYROID CYSTADENOMATA IN THE RAT

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SIX TEXT FIGURES AND THREE PLATES (TWENTY FIGURES)

It is well known that the various manifestations exhibited by the ultimobranchial body in different mammals have led to considerable controversy concerning the fate and significance of this structure. Outstanding interpretations of a number of investigators in this field have been recently reviewed (Van Dyke — in press). After this elusive structure becomes incorporated within the growing thyroid gland, it may undergo, depending upon the species, complete degeneration, transformation into thyroid-like parenchyma (by embryonic induction) or result in the formation of epithelial cysts which are usually lined by stratified squamous epithelium. Watzka ('33) has studied this structure in many mammals and suggests that, when the ultimobranchial body does not fuse intimately with thyroid parenchyma during embryonic development, it forms epithelial cysts; and when it is left outside the thyroid gland, it may give rise to cysts or abortive acini of mucoid character which are reminiscent of the kind of tissue from which the ultimobranchial body originated (general pharyngeal entoderm).

In many mammals this structure transforms more or less completely into thyroid-like tissue during embryonic development; presumably it persists as such. Rogers ('27) has very carefully described the changes involved during the transformation of ultimobranchial tissue into thyroid-like vesicles in the developing rat thyroid. Based on the assumption that the ultimobranchial body represents essentially indifferent tissue, with no strongly marked inherent potentialities, as originally suggested by Kingsbury ('14; also '35), Rogers came to the conclusion that the ultimobranchial body in the rat was induced by the adjacent embryonic thyroid parenchyma to transform into thyroid tissue. After birth it becomes impossible to distinguish this tissue from typical thyroid parenchyma.

In the embryonic human thyroid gland, Politzer and Hann ('35) could not decide whether gland-like remnants of the ultimobranchial body indicated developing thyroid parenchyma "or a functionally different gland tissue of the thyroid". Other investigators, in man at least, regard this portion of the fourth pharyngeal complex as possessing innate potencies for the formation of thyroid tissue and use the term "lateral thyroid" to designate it (Weller, '33; Norris, '37) — a term for which Kingsbury finds no justification ('39).

Not infrequently tumors of the cervical region in man have been ascribed to the ultimobranchial body when it fails to be incorporated within the thyroid gland (Leech, Clute and Smith, '28, and Ward, '40). These so-called "lateral aberrant thyroid tumors" are usually slow growing neoplasms. According to Leech, Clute and Smith they are most often of the cystadenomatous type with some papillary design, although Ward has observed many areas of adenocarcinoma where the tumor was invasive. Ward, regarding this tissue as a "lateral thyroid", suggests that such tumors arise laterally or inside the thyroid gland during adolescence or pregnancy in the female as the result of increased demand for thyroid function; remaining essentially benign until the proper stimulus for malignancy is furnished. When inside the thyroid gland solid cellular areas, typical of Langhans proliferating adenoma, may invade normal thyroid tissue; apparently functional areas sometimes appear in the midst of the tumor.

In young sheep the ultimobranchial body is usually represented in the thyroid gland by large multiple cysts lined by stratified squamous epithelium (Van Dyke, '43). They are regarded as arising congenitally due to the failure of the ultimobranchial body to fuse intimately with thyroid parenchyma during embryonic development. The epithelium of these cysts frequently produce cords or clumps of gland-like cells which invade the neighboring thyroid parenchyma in a manner that sometimes suggests a benign glandular neoplasm. Groups of these cells, most numerous in thyroids showing hyperplasia, form lobules which often differentiate plainly into thyroid-like follicles. The evidence was interpreted as indicating that cyst epithelium, indicative of ultimobranchial tissue which lacked inherent thyroid-forming potencies, is capable of producing undifferentiated ("fetal"-like) cells which may be induced by adjacent thyroid parenchyma to transform into thyroid-like vesicles.

Ultimobranchial cysts lined by stratified squamous epithelium have been observed, occasionally at least, in the thyroid gland of most mammals in which the ultimobranchial body has been studied. Such cysts were noted in the developing human thyroid gland by Herrmann and Verdun in 1899 and Groschuff in 1900. In atrophied thyroid glands of cretins and idiots Getzowa ('07) found epithelial cysts and solid cell masses which she regarded as "rests" of the ultimobranchial body. She also concluded that these cell masses were not comparable to thyroid parenchyma since they were composed of protoplasm-rich cells and, when lining cysts, often exhibited cilia. In studying squamous cell carcinoma of the thyroid gland Jaffe ('37) observed metaplasia of follicular epithelium to stratified squamous epithelium in four human thyroid glands of advanced age which exhibited sclerotic and inflammatory changes. This new epithelium was regarded as the source of the rare squamous cell carcinoma of the thyroid gland, but he states that, when the tumor is observed, the changes have usually advanced too far to allow any conclusion as to pre-existing lesions from which the tumor may have originated. Jaffe does not regard the origin of these tumors from "fetal rests" as likely, nor does he mention the possibility of an origin for these tumors from tissue that was originally derived from the ultimobranchial body.

Despite the frequent occurrence of this relatively indifferent tissue in the mammalian thyroid gland, no systematic study has been made to determine the ultimate fate of ultimobranchial tissue (i.e. — the nature of its cellular products; their relation to thyroid parenchyma and neoplasia of the postnatal thyroid gland) in an animal in which this structure undergoes more or less complete transformation into thyroid-like tissue during embryonic development. Such a study in man, in which the ultimobranchial body may undergo similar developmental changes (Weller, and Norris), is obviously a difficult though warrantable procedure.

In view of the normal cystic fate of this structure in young sheep, and the formation of ultimobranchial cysts in thyroid glands of vitamin A deficient rats (Van Dyke, '42), the writer proposes to discuss the frequency of such cysts in normal (stock) rats of various ages. The relationship of ultimobranchial cysts to thyroid parenchyma and to such obscure elements as "fetal"-like cells, which have been regarded by many investigators as the source of adenomata and the rarely encountered carcinomata in the human thyroid gland (Graham, '29, and Goetsch, '43), is discussed.

Review of the literature concerned with spontaneous tumors of the thyroid gland reveals the relative insusceptibility of normal rats to neoplasms in this organ. Bullock and Curtis ('30) state, "Of the many thousands of rats which have been examined postmortem only two developed thyroid tumors" (p. 88). One of these tumors was a carcinoma in a female 948 days old; the other was an adenoma in a female whose age ranged somewhere within 427–856 days. Both animals possessed primary tumors in other organs. In spite of the current view that true neoplasms of the thyroid gland are rare, evidence will be presented for the frequent development of thyroid tumors from ultimobranchial tissue in very old rats.

MATERIALS AND METHODS

Thyroid glands from 100 albino rats (sixty males and forty females), which had been maintained on a normal (stock) diet for variable periods, were examined grossly and microscopically.[1] Animals from forty-two separate litters including four dwarf rats were studied. These were placed in one of five age groups depending upon the age of the animal at the time it was sacrificed (table 1).

The entire laryngeal region together with the intact thyroid gland was excised from each animal and fixed in Bouin's' fluid, 10% formalin, Zenker's or Helly's fluid. Complete serial sections of these blocks of tissue were cut in the frontal plane at from 6 to 8 microns. Every tenth section was spread and mounted for preliminary examination. However, only that portion of each thyroid gland exhibiting ultimobranchial cysts and/or tumors was mounted in serial order for further study. All sections were stained with Harris's hematoxylin, and eosin; drawings were made with the aid of a camera lucida.

OBSERVATIONS

Apparently the ultimobranchial body of the rat is rarely left outside the thyroid gland during embryonic development. In the present study, however, the left lobe of the thyroid gland of one male rat (600 days old) was absent. Since the thyroid gland on the right side was about twice its normal size, it may be assumed that during embryonic development the thyroid anlage failed to bifurcate and came to lie wholly upon that side. The left ultimobranchial body was clearly demonstrable lying alone in the connective tissue (fig. 7). It was composed of a few tiny, irregularly shaped and densely stained vesicles which were clustered around one or two large cystic acini (not illustrated). These were lined by simple cuboidal or columnar epithelium with pynotic nuclei which, in the larger cysts, had secreted a colloid substance of poor quality. The whole "body" was enclosed in a dense mass of collagenous tissue containing numerous leucocytes. The thyroid gland was distinctly hyperplastic.

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[1] The writer wishes to express his appreciation to Prof. C. M. McCay, Director of the Animal Nutrition Laboratory at Cornell University, Ithaca, New York, for contributing generously to the specimens used in this investigation.

Ultimobranchial cysts. Cysts lined by stratified squamous epithelium (keratinizing), similar to ultimobranchial cysts described in young sheep, were identified in one or both lobes of the thyroid gland in 39% of the rats examined (twenty-two females and seventeen males). They were encountered in the thyroid gland of 15% of the rats in the normal young group, 34.5% of those in the adult group, 61.9% of the animals in the old group, and 58.3% of the glands belonging to the so-called old-age group. It was apparent that cysts were not only more frequent in females and in older animals but in rats whose thyroid glands exhibited parenchymal abnormalities. No thyroid cysts were observed in ten new-born rats studied (table 1).

Depending apparently on the age of the animal, these cysts may be single or multiple. They are more numerous in older rats where they may coalesce to form large cysts (figs. 9 and 16). Such cysts were restricted to the central region of each lobe of the thyroid gland in which they occurred, i.e., in the position normally occupied by the fetal ultimobranchial body (Rogers). Because of their location and intimate relation to adjacent thyroid parenchyma, separated from it only by a delicate connective tissue capsule, they are interpreted as arising (by metaplasia) from interfollicular cell clumps or thyroid-like follicles originally derived from the ultimobranchial body (figs. 6b; 8 and 9). Typically they lie in the same plane of section that includes the parathyroid gland (III); in one instance the cyst was connected to it.

As in young sheep, although to a lesser extent, large, clear (gland-like) cells with relatively agranular cytoplasm and a moderately chromophilic nucleus arise in the wall of these cysts. They resemble a fetal type of cell and are regarded, therefore, as more or less undifferentiated. These cells take origin from certain germinative cells in the stratified squamous epithelium and during the process of their transformation become enlarged and difficult to stain. Arising singly or in clumps they ultimately come to lie at first between the cyst wall and its connective tissue capsule where they form clusters or small vesicles which resemble young thyroid follicles. They do not grow actively away from the cyst, but appear to be removed as the result of a more or less passive process brought about largely by their individual hypertrophy (figs. 1 and 12).

Thyroid parenchyma. Of the 61% of the thyroid glands which did not contain epithelial cysts, 32 were classified as normally active, 14 as exhibiting varying degrees of hyperplasia, 8 as relatively inactive (resting), 2 as atrophic; and 5 as representing different stages of follicular hypertrophy similar to colloid goiter. Table 1, however, summarizes only those parenchymal alterations occurring in thyroids containing ultimobranchial cysts. It shows both the distribution of these cysts with regard to the sex and age of rats; and a correlation between cyst formation and the production of gland-like cells, and the kind of altered parenchymal manifestation. It will be noted that epithelial cysts seldom occur in comparatively normal thyroid glands. They are frequently encountered (and with greater productivity of gland-like cells) in thyroids in which the parenchyma appears to have been hyperplastic, unstable (degenerating), or atrophic — i.e., in those thyroids which were more active or less active than normal. In the following paragraphs representative thyroid glands containing epithelial cysts in each age group will be briefly described.

In the young group of twenty rats the thyroid gland of three contained ultimobranchial cysts (table 1). In each of these, cysts were few, small and thick-walled (fig. 9). Since the very existence of such cysts is usually incompatible with experimentally induced thyroid overactivity (unpublished), the relative lack of ultimobranchial cysts in young rats may be due to physiologic hyperthyroidism of youth. Figure 9 illustrates severe hyperplasia of the thyroid gland in a young male rat 70 days old; showing considerable destruction of follicular epithelium characterized by many pycnotic nuclei. A large, clear gland-like cell can be seen arising from the basal layer of cells in each cyst.

The thyroid gland of all dwarf rats studied (4) contained ultimobranchial cysts. Figure 8 shows a typical ultimobranchial cyst in the thyroid gland of one of these 25-day-old dwarf rats. Thyroid follicles were diminutive (compare with fig. 10 which is of lower magnification) and possessed thin, densely stained epithelium. Colloid, when present, stained faintly with basic dyes. Despite the presence of resorption vacuoles in some follicles such glands are regarded as hypoplastic and therefore incompetent. Whether the ultimobranchial body failed to be induced by adjacent thyroid parenchyma to transform into thyroid-like tissue during embryonic development and thus persisted as epithelial cysts (as in sheep), or whether these cysts arose secondarily from ultimobranchial tissue after birth cannot, at present, be determined. Similar cysts, however, invariably arise in the thyroid gland of young rats kept on a vitamin A deficient diet (Van Dyke, '42). Since

TABLE 1

This table illustrates the frequency of ultimobranchial cysts in the thyroid gland, and their distribution with regard to age and sex, of 100 albino rats. The state of thyroid parenchyma in those glands containing cysts is indicated. Asterisks (–***) represent relative extent of cyst formation; those in parentheses contributed gland-like (''fetal'') cells to adjacent thyroid parenchyma. R.I., relatively inactive; D., degenerative; T., tumor.*

RATS STUDIED	AGE	SEX	% CYSTS	RIGHT	LEFT	THYR. PAR.
	(days)		Newborn			
10	7			0	0	Normal (active)
			Young			
4	25	♂	100. (dwarf)	(*)	0	Hypoplastic
		♂		*	*	Hypoplastic
		♀		(*)	(*)	Hypoplastic
		♀		0	*	Hypoplastic
20	15–80	♀	15.	0	*	Normal (R.I.)
		♀		(**)	(***)	Hyperplastic
		♂		(**)	(**)	Hyperplastic
			Adult			
29	115–300	♂	34.5	*	0	Normal (R.I.)
		♀		*	0	Atrophic
		♂		0	(**)	Atrophic
		♂		*	0	Atrophic
		♂		*	0	Atrophic
		♂		*	(**)	Atrophic
		♂		*	*	Unstable (D)
		♂		0	(**)	Unstable (D)
		♀		(***)	(***)	Hyperplastic
		♀		(***)	(***)	Hyperplastic
			Old			
21	600–650	♀	61.9	*	*	Normal (R.I.)
		♀		**	0	Atrophic
		♀		(**)	0	Atrophic
		♀		(*)	(*)	Atrophic
		♂		*	0	Atrophic
		♂		*	(**)	Atrophic
		♂		0	*	Atrophic
		♀		(*)	0	Unstable (D)
		♀		(**)	(**)	Unstable (D)
		♀		0	(**)	Unstable (D)
		♂		0	*	Unstable (D)
		♀		0	(**)	Hyperplastic
		♀		(**)	(**)	Hyperplastic
			Old-age			
16	801–906	♂	58.3	(*)	0	Atrophic
		♂		(*)	(*)	Atrophic
		♂		0	*–T	Atrophic
		♀		0–T	0	Atrophic
		♀		*–T	0	Atrophic
		♀		(*)–T	0	Atrophic
		♀		*–T	0	Atrophic
		♀		*–T	0	Atrophic
		♀		0	*–T	Atrophic
		♀		*–T	*–T	Atrophic

Wendt ('35) found that blood plasma of cretins contains little vitamin A due to failure of conversion of carotene to vitamin A in hypothyroidism, these cysts in dwarf rats may be due to relative deficiency of this vitamin. Ultimobranchial cysts in hypoplastic thyroid glands also produce gland-like cells that give rise to thyroid-like vesicles (fig. 5).

Ultimobranchial cysts were encountered in ten of twenty-nine thyroids removed from adult rats (table 1). Figure 1 shows a section of the thyroid in a female 290 days old containing a cyst. An adjacent section of the same cyst is illustrated in figure 12. Large, gland-like cells may be identified in the cyst epithelium, or between the cyst and its connective tissue capsule in these figures. Thyroid parenchyma, despite evidence of colloid resorption, appears to have undergone a degeneration that has led to partial destruction of thyroid follicles (fig. 12).

Figure 10 is a section through an enlarged thyroid gland of a male rat 300 days old and shows a rather typical picture of early diffuse parenchymatous hyperplasia. A small thyroid-like vesicle and clumps of gland-like cells that appear to have arisen from the cyst wall lie between its epithelium and the connective tissue. Figure 3 shows a similar ultimobranchial cyst in an overactive thyroid gland of a female 200 days old. In figures 2, 11 and 13, different sections of the same cyst in the hyperplastic thyroid gland of a female 250 days old are represented. Numerous gland-like cells not only arise from cyst epithelium in such overactive thyroid glands, but appear to be transformed into typical thyroid tissue (figs. 3 and 13). Study of these figures will show that, where such metamorphosed basal cells have become dis-

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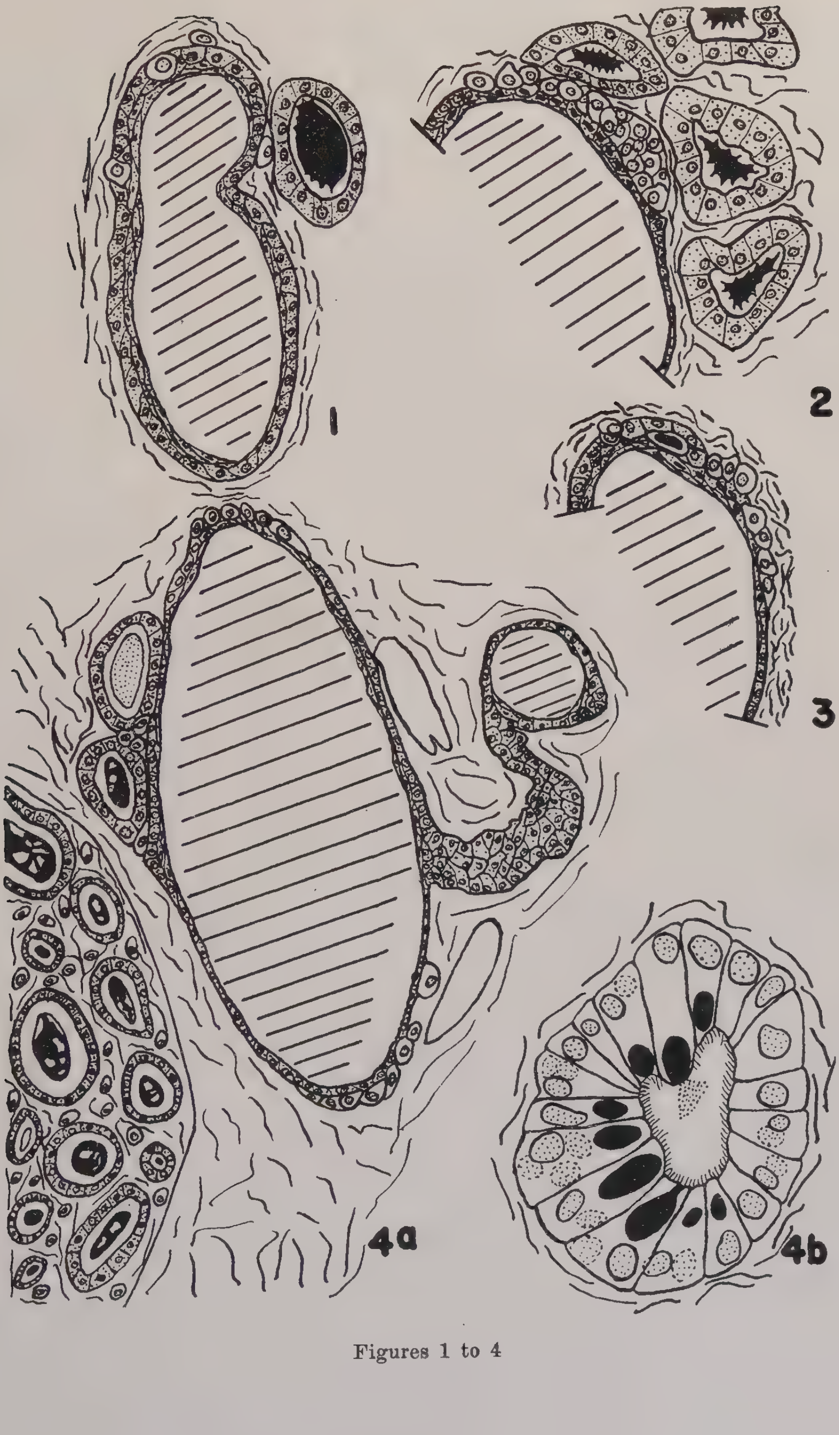
Fig. 1 Cyst lined by stratified squamous epithelium in an unstable thyroid gland (degenerating) of an adult female rat (fig. 12). Note clear (unstained) gland-like cells in basal layer of the epithelium, and between the cyst wall and its connective tissue capsule. × 220.

Fig. 2 Similar cyst in the hyperplastic thyroid gland of an adult female (figs. 11 and 13). Note that where many gland-like cells are forming from basal cells of the cyst, the portion of the epithelial wall left intact is thin and represents largely the most superficial layer of (keratinizing) cells (compare with figs. 3 and 4a). × 220.

Fig. 3 Ultimobranchial cyst found in the hyperplastic thyroid gland of an adult female rat. Note many gland-like cells and one small thyroid-like follicle which lie between the cyst wall and adjacent connective tissue. × 220.

Fig. 4a Cyst found in the atrophic thyroid gland of an old female rat. Note the following: atrophic thyroid parenchyma on the left, growth extension of cyst on the right, formation of gland-like cells from the basal layer of cells at both ends of cyst, and two thyroid-like follicles of probable cyst origin separated from thyroid parenchyma by connective tissue. Upper vesicle is atypical and appears to contain mucoid secretory products (see also fig. 17). × 220.

Fig. 4b Mucous-like acinus derived from gland-like cells of ultimobranchial cyst origin in an atrophic thyroid gland of an old female. Note resemblance of these cells to goblet cells with cilia, one of which is extruding its products of secretion. × 440.

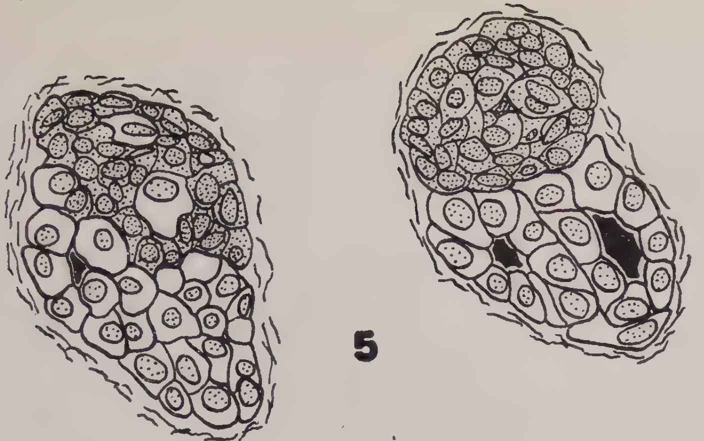


Figures 1 to 4

placed from the epithelium, the part of the cyst wall left intact may be temporarily deficient and appears thin-walled.

Thirteen thyroid glands out of a total of twenty-one in the old group exhibited ultimobranchial cysts (table 1). Figures 14 and 15 illustrate the atrophic thyroid parenchyma in two rats (female and male respectively) approximately 600 days old. In spite of evidence of colloid resorption in both glands, the parenchyma shows many degenerative changes. Their epithelial cells are small, densely stained (with pycnotic nuclei) and exhibit an instability that has resulted in considerable follicular destruction. Gland-like cells derived from cysts in such thyroid glands apparently do not organize to form thyroid-like follicles (fig. 15, lower arrow). The atrophic thyroid parenchyma of two female rats 650 days old containing ultimobranchial cysts is shown in figures 16 and 17. In both sections thyroid follicles are variable in size, thin-walled and densely stained. Colloid is reduced and appears retracted from the epithelium of these follicles. Interfollicular cells were numerous in both thyroid glands. In figure 4, which is a section of the thyroid adjacent to that in figure 17, gland-like cells have taken origin from the epithelium at both ends of the cyst. On the left side two thyroid-like follicles which appear to have arisen from cells derived from cyst epithelium may be clearly identified. They are distinctly isolated from neighboring thyroid parenchyma by connective tissue. The lower vesicle has the appearance of a typical thyroid follicle. The upper, like those shown below the cyst in figure 17, represents an atypical follicle containing granular debris mixed with basophilic globules. Evidently such thyroid-like vesicles (in under-active or atrophic thyroid glands) may occasionally undergo metaplasia, resulting in additional cysts lined by stratified squamous epithelium or even mucous-like acini (figs. 17 and 4b). Figure 4b suggests a rather typical mucous acinus composed of goblet cells with cilia. It was found adjacent to an ultimobranchial cyst in the atrophic thyroid gland of a female rat 640 days old.

Thyroid adenomata. Of the sixteen very old rats studied (801–906 days old), there were ten females and six males. These represented the surviving members of ten separate litters. In ten rats of this group (seven females and three males) the thyroid parenchyma exhibited variable but distinct characteristics of senile atrophy. Ultimobranchial cysts were identified in nine of these atrophic thyroid glands (six females and three males). The character of these glands has been interpreted as presenting histological evidence of hypothyroidism in aged rats. The following criteria distinguish these thyroid glands as a whole. Each gland was small, relatively avascular and contained a



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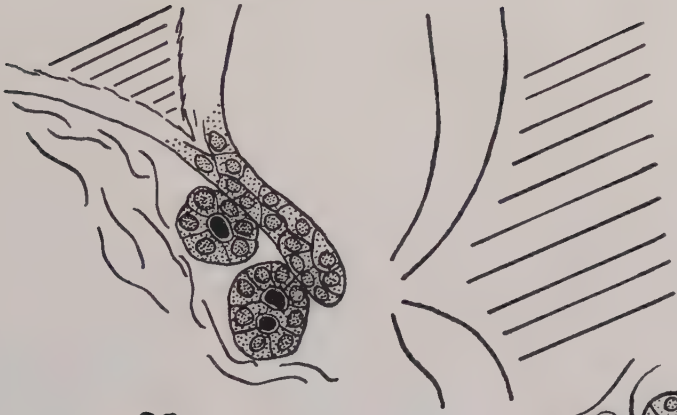
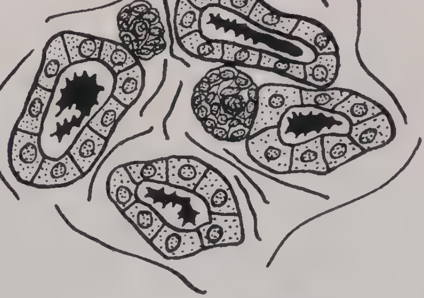


6a



6b

Fig. 5 Tangential sections of two cysts in the hypoplastic thyroid gland of a 25-day-old dwarf rat. Note clear, undifferentiated type of cells in the epithelium of these cysts, and clumps of similar gland-like cells or small thyroid-like vesicles outside the cyst epithelium (stippled) but within the connective tissue capsule of the cyst. × 400.

Fig. 6a High power drawing of transitional zone between ultimobranchial cyst and thyroid adenoma shown in figure 25 (arrow). Two tiny thyroid-like vesicles, undoubtedly derived from the epithelium of this thin-walled cyst, are shown. They stain densely and possess pycnotic nuclei. Such vesicles are regarded as the most probable source of these thyroid adenomata in old rats. × 400.

Fig. 6b Showing origin of ultimobranchial cysts from interfollicular cell clusters. Note their position and size with respect to adjacent hyperplastic thyroid follicles (same thyroid as shown in fig. 9). × 400.

considerable amount of connective tissue. Thyroid follicles were small, irregular in size and shape, thin-walled and composed of cells which were reduced in size and frequently contained pycnotic nuclei (figs. 19 and 25). Mitosis of epithelial cells was rare. In many follicles the colloid was absent or reduced; frequently it exhibited a greater affinity for basic stains or appeared less dense than normal (fig. 25). There was little evidence of colloid resorption. In many glands the colloid contained densely staining (basophilic) globules which suggested droplets of mucus or calcium deposits (figs. 19 and 20). Interfollicular cells which were characteristic of some glands appeared to have resulted from follicular destruction. In those thyroid glands containing large tumors the parenchyma had undergone almost complete pressure atrophy (figs. 21, 23 and 26).

An early manifestation of thyroid neoplasm, lying near a cyst in the left lobe of the thyroid gland, represented the only instance in which a tumor was identified in male rats (fig. 18). The thyroid gland of seven old-age female rats, however, contained a total of eight individual thyroid tumors. Without exception, each tumor occurred in an atrophic thyroid gland and, with only one exception, was found closely associated with, or attached to, an ultimobranchial cyst (table 1). These ''new growths'' have been classified as relatively slow growing (benign) adenomata. In certain regions of the same neoplasm, they may exhibit a different pattern. The smaller (younger) tumors, except for the dense staining character of their epithelial cells, resemble more closely the normal architecture of the thyroid gland. Figures 18 and 20 show two early stages in the development of these adenomata. The larger (older) tumors, although simulating thyroid parenchyma, have lost much of their thyroid-like structure; exhibiting both cyst formation and a papilliferous design (figs. 22, 23, 24 and 26).

In each case the tumor formed a single nodule lying in or near the center of the lateral lobe of the thyroid gland — in the region where tissue derived from the ultimobranchial body would be expected to lie (fig. 24). The character of their cellular constituents was identical to that found in the ultimobranchial body lying outside the thyroid gland in one male rat. Such tumors were composed of densely staining columnar epithelial cells, possessing pycnotic nuclei, which in most parts of the tumor were arranged to form cysts of irregular shape and size (fig. 26). Usually there was a large central cyst with peripheral extensions that had given rise to thyroid-like vesicles of similar cellular character; in a manner as shown in figure 18, causing the tumor to increase in size (see also figs. 21 and 23). Despite their degenerative character, they appear to have been actively functioning as aberrant thyroid tissue. Not only did they secrete considerable quantity of strongly eosinophilic colloid that showed evidence of resorption, but they were also the most highly vascularized portion of the thyroid gland.

The proximity of ultimobranchial cysts to thyroid adenomata is illustrated in figure 21. Since these cysts in old rats are usually thin-walled, composed largely of the more superficial (keratinizing) cells of the epithelium, it appears that most of the basal cells which were capable of being transformed into typical thyroid-like tissue had been previously removed. Certain gland-like cells derived from such epithelial cysts, however, were undoubtedly responsible for the growth of these thyroid adenomata. Figure 25 is an example of a thyroid tumor which is attached to an extension of a thin-walled ultimobranchial cyst, illustrating clearly the source of origin of cells forming these tumors. The transitional area between cyst and adenoma is represented at a higher magnification in figure 6a. Two densely stained thyroid-like follicles, apparently derivatives of the cyst wall, are shown in this figure. Their epithelial cells possess pycnotic nuclei and have secreted a colloid that stains deep red with eosin, similar to colloid produced by tumor cells. Similar densely staining vesicles have been observed by the writer in young sheep. As in sheep, these atypical gland-like cells are contributed to thyroid parenchyma by the more superficial cells of the cyst epithelium, i.e., those epithelial cells which might be regarded, by some investigators, as irreversibly differentiated toward keratinization. Such cells are regarded as the most probable source of thyroid adenomata in the atrophic thyroid gland of the rat and will be discussed.

DISCUSSION

Certain opinions concerning the nature and fate of the ultimobranchial body and the origin of thyroid cystadenomata, in the rat at least, appear justified from the evidence presented in this study. There can be little doubt that these epithelial cysts represent ultimobranchial tissue, since they are identical in all respects to ultimobranchial cysts known to exist in the thyroid gland of other mammals (cat, sheep and man) and, in the rat, lie consistently in or near the center of each lobe of the thyroid gland. It is very improbable that the highly specialized parenchyma of the thyroid could dedifferentiate and undergo metaplasia with the resultant formation of cysts lined by stratified squamous epithelium. Since there is no reason to suppose that only a few selected thyroid follicles would undergo this change and not become involved in the parenchymal degeneration that usually affects the gland as a whole, the tissue from which these cysts arose must have been of different character. This viewpoint, supported by the evidence, purports to deny the existence in mammals of a ''lateral thyroid'' which possesses inherent potentialities for the formation of thyroid tissue; since ultimobranchial tissue, unlike the thyroid parenchyma derived from the midline of the pharyngeal floor, is not irreversibly differentiated into thyroid tissue during development. The writer, on the basis of his studies, as well as those of Kingsbury and Watzka, regards the ultimobranchial body in mammals as essentially indifferent tissue which may retain only those potencies that characterize, in general, the region from which it arose.

The time at which the thyroid gland becomes functionally active in the embryo may be a factor in determining the fate of the ultimobranchial body. In the rat the period at which transformation of this tissue begins coincides closely with the time at which the embryonic thyroid gland becomes functional. Gorbman and Evans ('43) point out that the functional ability of rats to store iodine begins in the eighteenth to nineteenth day embryo and may be correlated with the first differentiation of follicles with lumina. Reference to Rogers' publication reveals that, although the period of most active proliferation of ultimobranchial tissue occurs prior to sixteen and a half days, ''the real transformation . . . begins to manifest itself at seventeen days'' (p. 356).

The writer would suggest that in those mammals like the rat where intimate fusion of the ultimobranchial body with thyroid parenchyma occurs, the ability of the adjacent thyroid tissue to induce this structure to transform into thyroid-like tissue during embryonic development, and maintain its integrity after birth, depends largely on the inducing power provided by normally active thyroid parenchyma which subsequently holds this tissue in bond. Epithelial cysts in the thyroid gland of dwarf rats, therefore, have been interpreted as a characteristic expression of ultimobranchial tissue in the presence of underactive (hypoplastic) thyroid tissue, which is neither conducive to the transformation of this structure into thyroid-like follicles during embryonic development nor the maintenance after birth of such vesicles previously formed.

Since these cysts, which may be produced experimentally in rats fed a vitamin A deficient diet, yield numerous gland-like cells that may lead to exhaustion of the cyst when the animal is injected with thyrotropic hormone (as previously mentioned), the production of such cells in hyperplastic thyroids of stock rats has been interpreted as evidence for the ability of active thyroid parenchyma to induce these cysts to transform into thyroid-like tissue. They may persist, however, as thin-walled cysts composed only of the more superficial (keratinizing) cells of the original epithelium. As such they may be a source of thyroid neoplasm during hyperplasia, as appears to be the case in some atrophic rat thyroids. What the ultimate fate of gland-like cells removed from ultimobranchial cysts during thyroid hyperplasia may be is not known. In sheep and rats they appear identical to interfollicular cells observed in various mammals by many investigators.

Since these cysts also occur in the human thyroid gland, the possibility of thyroid adenomata arising near the superior parathyroid gland, similar to those in the rat, cannot be excluded or even regarded as remote. It seems plausible to suggest that, the ''fetal''-like cells regarded by Graham and Goetsch as the source of some adenomata in the human thyroid gland may, indeed, have been derived from ultimobranchial tissue — possibly from ultimobranchial cysts originally formed as the result of an atrophic or underactive state of thyroid parenchyma. Such cysts, being more or less transient in hyperplastic thyroid glands, may not be readily identified with tumors in an overactive thyroid gland. However, their cellular products, many of which are ''fetal''-like in character, may persist indefinitely; and in the human thyroid gland may prove to be of particular significance to the pathologist.

The fact that ultimobranchial cysts occurred most often in those rat thyroids exhibiting parenchymal degeneration, and particularly in the atrophic glands of older animals, substantiates the belief that the integrity of thyroid-like follicles derived originally from ultimobranchial tissue depends on the normalcy of thyroid parenchyma. This viewpoint is also supported by the observations of Getzowa and Jaffe which were previously mentioned. The evidence has been interpreted as indicating that degeneration or senile atrophy of thyroid parenchyma has resulted in the removal, from those thyroid-like vesicles derived from ultimobranchial tissue, of the inhibitory influences preventing individual expression of this tissue that were resident in normally active thyroid parenchyma. As a consequence these follicles underwent dedifferentiation, followed by retrogressive changes that led to the expression of inherent potencies possessed by ultimobranchial tissue — namely, the formation of cysts lined by stratified squamous epithelium of the mucous type. Therefore, gland-like cells produced by these cysts, are regarded for the most part as an expression of the inherent growth potencialities of this epithelium; forming new cells of mucoid character which, although not induced by the underactive thyroid parenchyma to transform into typical thyroid-like follicles, give rise to atypical thyroid vesicles that secrete a basophilic colloid or to mucous-like acini (figs. 17;

4a and b). It should be recalled that Watzka noted the formation of mucous-like acini from ultimobranchial tissue outside the influence of the thyroid gland. The writer has also observed a similar manifestation of this tissue within the thyroid gland of sheep.

The thin character of ultimobranchial cysts in atrophic rat thyroids containing tumors suggests that they have been stripped of all potential, typical gland-like cells. Such cells might be regarded as a latent source of some thyroid adenomata. In these senile rats, however, it seems most probable that these undifferentiated or ''fetal''-like cells have been induced to transform into thyroid-like vesicles despite the atypical character of thyroid parenchyma. The writer would suggest further that, thyroid adenomata in aged rats develop from densely staining thyroid-like vesicles whose atypical gland-like cells take origin from those superficial epithelial cells retained by thin-walled ultimobranchial cysts, i.e., from small cells with pycnotic nuclei that might be regarded as differentiated toward degeneration. These cells would be closest to thyroid parenchyma; indeed, atypical thyroid vesicles, derived probably from this source, were found intimately associated with some tumors (fig. 25). Such cells possess characteristics similar to those of tumor cells, and have also been identified in sheep where they plainly arise from the more superficial epithelial cells of these cysts. In young sheep they form irregularly shaped cysts, lined by densely staining columnar epithelium with pycnotic nuclei, that are identical to the incipient thyroid tumor of the rat shown in figure 18.

Without forcing, these would seem to be the most likely cells to undergo neoplastic change, resulting in an adenoma that is apparently attempting to compensate for the underactivity of atrophic thyroid parenchyma. Unlike the undifferentiated gland-like cells whose fate is dependent upon the ability of atrophic thyroid tissue to induce them to transform into thyroid-like parenchyma, and which in compensating for the underactivity of the gland would conceivably undergo only hyperplasia; the densely staining cells, which have already been partially differentiated toward keratizination, cannot be induced to form typical thyroid parenchyma but undergoing growth result in the formation of aberrant thyroid tissue.

CONCLUSIONS

1. Ultimobranchial cysts lined by stratified squamous epithelium may be frequently encountered in the thyroid gland of albino rats. Such cysts characterize the unstable (degenerating) and atrophic thyroid gland, and are most frequent in female or older rats.

2. Large gland-like cells, "fetal" in character, which are derived from the basal layer of cells in the epithelium of these cysts come to lie between the cyst wall and its connective tissue capsule where they form clumps or small vesicles which resemble young thyroid follicles.

3. The production of these conspicuous gland-like cells is most apparent in hyperplastic thyroid glands where they appear to be induced by the adjacent active thyroid tissue to transform into typical thyroid-like parenchyma; and in atrophic thyroid glands where such gland-like cells may transform into thyroid-like follicles or vesicles that may differentiate into mucous-like acini.

4. Spontaneous adenomata, exhibiting both cyst formation and a papilliferous design, are not infrequently encountered in the atrophic thyroid gland of very old rats. They take origin from certain densely staining, atypical gland-like cells derived from the epithelium of ultimobranchial cysts. The mode of origin and significance of cysts, "fetal"-like cells, and tumors is briefly discussed.

5. The evidence obtained in this study has been interpreted as indicating that the ultimobranchial body, which lacked inherent thyroid forming potentialities, may be induced by the embryonic thyroid gland to transform into thyroid-like tissue during embryonic development, but that these follicles may transform into cysts lined by stratified squamous epithelium after birth; that these cysts usually arise after birth in response to underactivity or an atrophic state of the thyroid parenchyma; and that certain gland-like cells produced by the epithelium of these cysts may give rise to highly vascular, compensating thyroid cyst-adenomata in atrophic thyroid glands of aged rats.

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PLATE 1

EXPLANATION OF FIGURES

7 Section through the isolated left ultimobranchial body of an old male rat. Left lobe of the thyroid gland was absent. Note clumps of densely stained vesicles without conspicuous cavities. × 110.

8 Small ultimobranchial cyst lined by stratified squamous epithelium in the right lobe of the thyroid gland of a male (dwarf) rat 25 days old. Thyroid follicles are unusually small, thin-walled and possess densely stained nuclei. Colloid had a greater affinity for basic stains. Thyroid parenchyma exhibits hypoplasia. × 200.

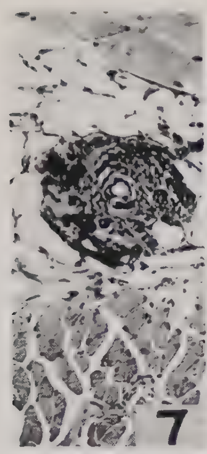
9 Similar cysts in the left lobe of the thyroid gland of a young male rat. Thyroid parenchyma shows severe hyperplasia. Note formation of gland-like cells from the basal layer of cells in both cysts (arrows). × 185.

10 Ultimobranchial cyst in the left lobe of the thyroid gland of an adult male rat. Thyroid parenchyma shows an early stage of diffuse parenchymatous hyperplasia with considerable loss of colloid. Note the small clump of cells and the small thyroid-like vesicle lying between the cyst wall and its connective tissue capsule (arrow). × 110.

11 Cyst lined by stratified squamous epithelium in the right lobe of the thyroid gland of an adult female rat. The thyroid gland exhibits hyperplasia. Note the clump of gland-like cells that have originated from the cyst wall, and the thin layer of superficial (keratinizing) cells which are left intact at this point. × 185.

12 Similar cyst in the right lobe of the thyroid gland of an adult female rat. Thyroid follicles show degeneration of epithelial cells. One gland-like cell is clearly represented in the basal layer of the epithelium of the cyst. × 185.

13 Section from the same thyroid gland as shown in figure 11. Note recently formed gland-like cells in the region of the lower arrow and the small thyroid-like vesicle composed of similar cells at the upper end of cyst. × 240.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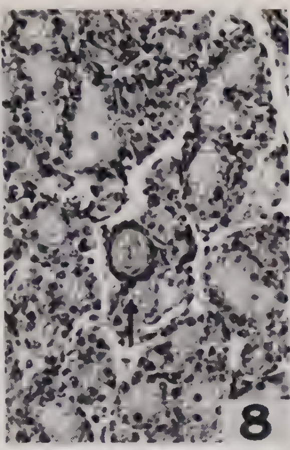
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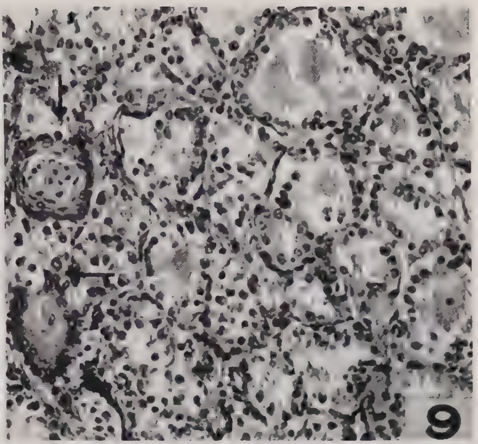
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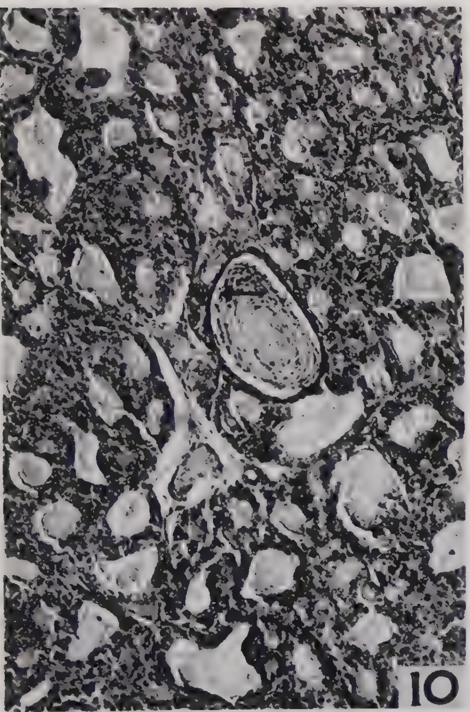
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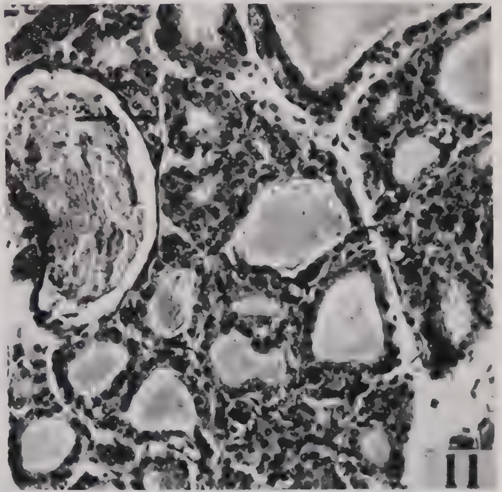
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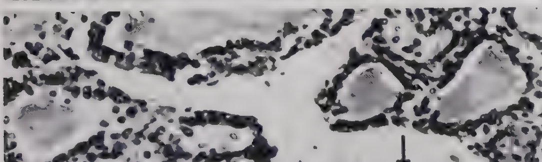
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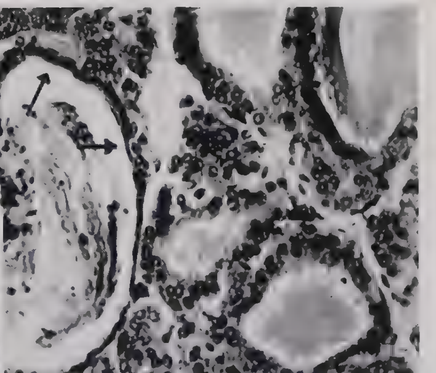
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PLATE 2

EXPLANATION OF FIGURES

14 Small ultimobranchial cyst in the right lobe of the thyroid gland of an old female rat. Note severe atrophic character of thyroid parenchyma; resulting in follicular disintegration. A cluster of gland-like cells may be seen in the epithelium of this cyst. × 150.

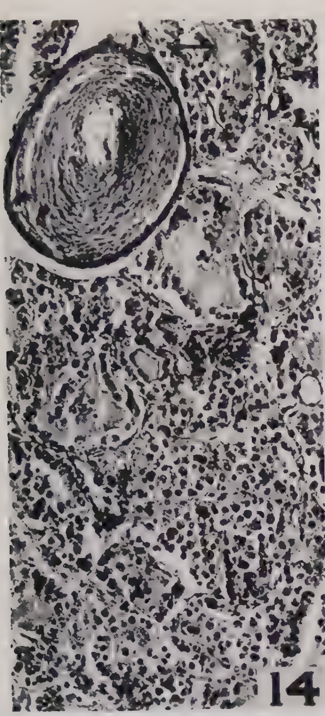
15 Cyst composed of a thin layer of stratified squamous epithelium in the left lobe of the thyroid gland of an old male rat. Thyroid parenchyma shows an atrophic condition similar to that in figure 14. Gland-like cells derived from the epithelium of the cyst are shown by arrows. The clump of cells at the lower end of the cyst lies between the cyst wall and the adjacent connective tissue. × 100.

16 Section of the right lobe of the thyroid gland of an old female rat, showing an ultimobranchial cyst near the center of the gland. Gland-like cells, although not well illustrated at this magnification, also occur in the wall of this cyst. The small thyroid-like follicles situated below the cyst may have had their origin from the epithelium of this cyst. Thyroid parenchyma is atrophic. × 100.

17 Section through the right lobe of the thyroid gland of an old rat, illustrating an ultimobranchial cyst with a growth extension. Upper arrow shows a small cyst which may have arisen by metaplasia from a thyroid-like follicle derived from gland-like cells produced by the major cyst. Between the major cyst and the lower arrow is a group of small thyroid-like vesicles derived probably from gland-like cells of the adjacent cyst (see also figure 4a which is another section of this cyst). These are atypical follicles containing a dust-like and more basophilic type of colloid. Thyroid parenchyma is atrophic. × 100.

18 Section through the left lobe of the thyroid gland of an old-age male rat 812 days of age, showing an incipient adenoma. Thyroid parenchyma is regarded as atrophic. Note the evaginations of the wall of the larger vesicle, forming additional vesicles of the same type, and the dense staining character of its epithelial cells. Colloid produced by these cells stains densely with eosin and shows evidence of resorption. × 100.

19 Section through the left lobe of the thyroid gland in an old-age female rat 854 days of age. Note the heavier stained, compact mass of cells in the center of the figure. It has not been interpreted as an additional kind of adenoma since such restricted areas may represent localized hyperplasia. Note the conspicuous, densely stained (basophilic) globules within the colloid of the follicles. Thyroid parenchyma is atrophic. × 110.



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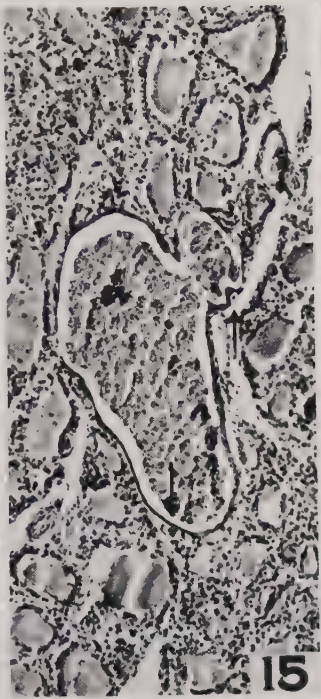

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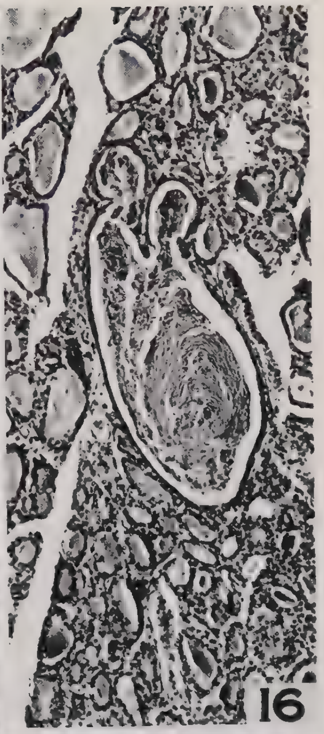

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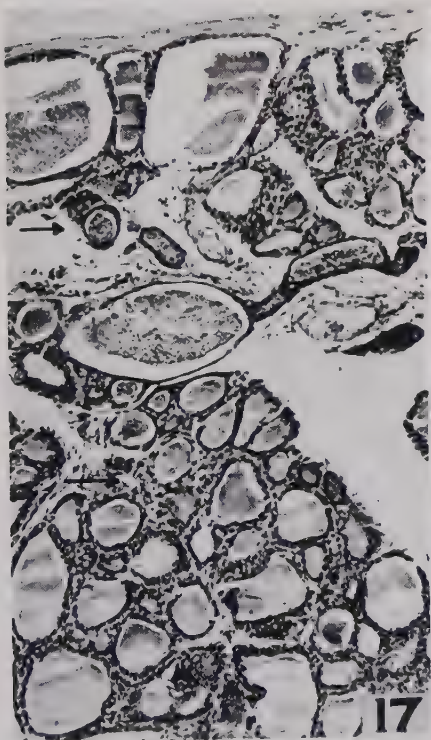

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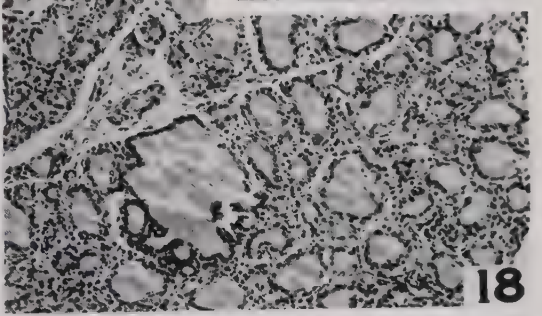

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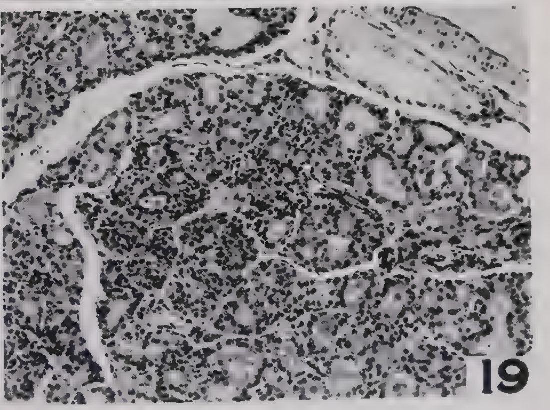

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PLATE 3

EXPLANATION OF FIGURES

20 Section of the right lobe of the thyroid gland illustrated in figure 19, showing a relatively early stage in the development of a thyroid tumor. Note the severe atrophic state of thyroid parenchyma. × 75.

21 Section through the right lobe of the thyroid gland of an old-age female rat 860 days of age, showing the proximity of an ultimobranchial cyst and a thyroid tumor. Note thin character of the epithelium of the cyst and the papilliferous pattern of the adenoma. Desquamated epithelial cells can also be seen in the cystic areas of the tumor. Thyroid parenchyma exhibits extreme atrophy. × 65.

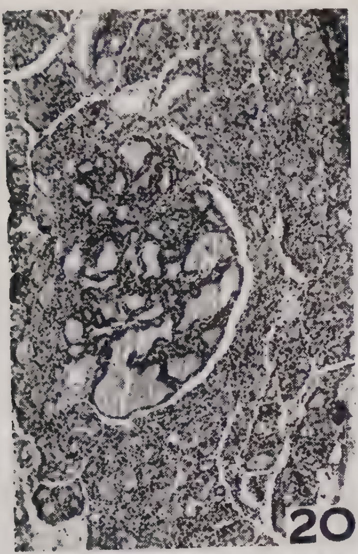
22 Section of a thyroid adenoma in the left lobe of the thyroid gland of a female 830 days old, showing the papillary design found in some portions of these tumors. × 110.

23 Low power view of an adjacent section of the thyroid tumor shown in figure 21. Note parathyroid gland (III) in upper left hand corner and the cystic character of the tumor in this atrophic thyroid gland. This was the largest tumor found in these rats. × 15.

24 Low power view of the right lobe of the thyroid gland in an old-age female rat 880 days old. Note position of the tumor in the central and medial part of the thyroid gland, and the space in the center of the tumor where colloid dropped out during sectioning of the gland. Parathyroid gland on the left. Thyroid parenchyma is atrophic. × 15.

25 High power view of the region of transition between the tumor shown in figure 24 and an ultimobranchial cyst. Such tumors therefore arise from ultimobranchial cysts in the thyroid gland of the rat. The region at the arrow is represented under higher power in figure 6a.

26 Section through the right lobe of the thyroid gland of a female rat 960 days of age. Note the irregularly shaped cystic follicles containing products of secretion in this tumor, and the papilliferous character of its epithelium. Thyroid parenchyma at lower left corner of this figure exhibits almost complete atrophy. × 50.



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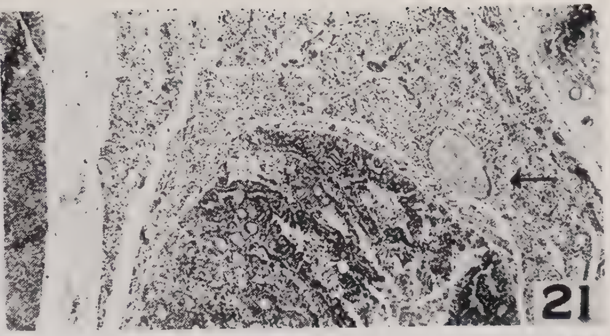

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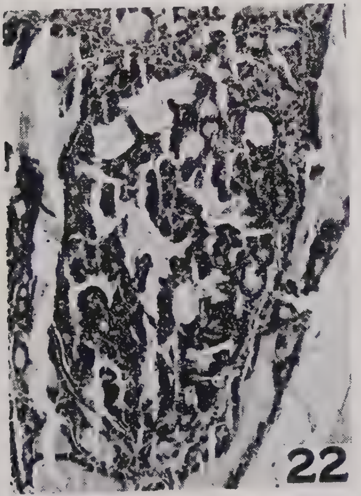

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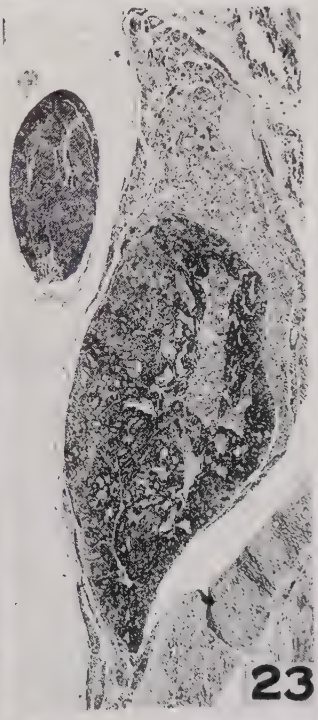

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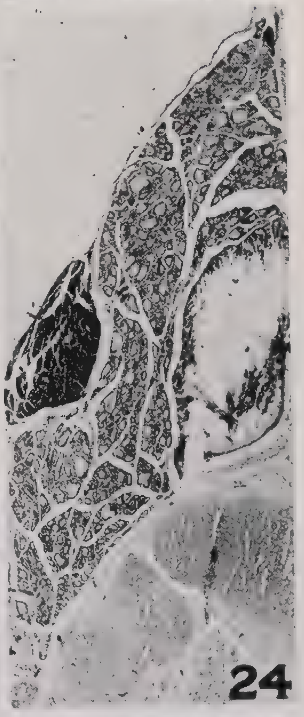

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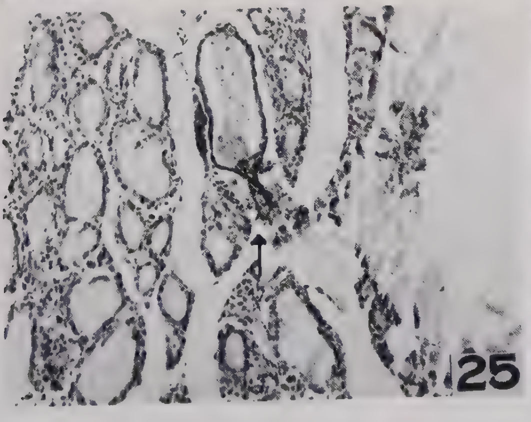

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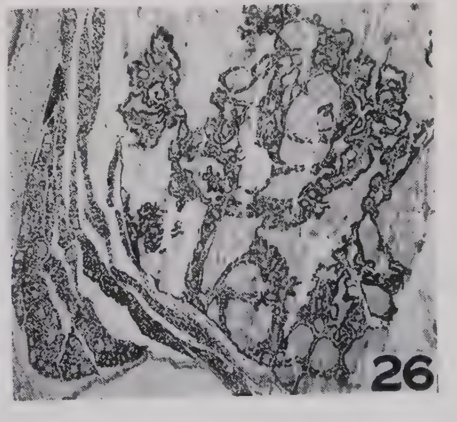

26

OVARIAN FUNCTION IN HYPOPHYSECTOMIZED RATS

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ELEVEN FIGURES

It is well known that after hypophysectomy in the rat estrous cycles cease and the ovary undergoes marked atrophy. Within 2 to 4 days the large ovarian follicles show signs of degeneration and no new ones develop beyond the early antrum stage (Smith, '30), while within 12 days the theca cells become transformed into plasma or wheel cells (Selye, '33). On the other hand, the corpora lutea regress very slowly (Smith, '30). In this the rat differs markedly from the mouse in which atrophy of the corpora lutea is particularly rapid (Selye, Collip and Thomson, '33). This slow rate of involution in the rat is not affected by the administration of progesterone or follicle stimulating hormone, but treatment with luteinizing preparations of the anterior pituitary cause their rapid disappearance (Bunde and Greep, '36).

In view of these observations Nelson and Merckel ('37) were surprised to find that "dehydroandrosterone" caused almost continuous vaginal cornification for 3 weeks in a not-spayed hypophysectomized rat just as it did in the normal, while in the spayed animal at the same dosage level, the vaginal smear was continuously diestrous. The ovary appeared to be able to transform this substance into a more folliculoid type of compound without the intermediary of the pituitary. Parkes and Zuckerman ('38) observed a similar response to testosterone after hypophysectomy and this was confirmed by Noble ('39) with testosterone propionate. The latter author found that cornification is not elicited in spayed or immature hypophysectomized rats but occurs if precocious puberty is induced with gonadotropic extracts before hypophysectomy. Thus the presence of a corpus luteum appears to be essential although it need not be recently formed since vaginal cornification was observed even if injections were commenced only 10 days after hypophysectomy. In the rat testosterone induces only transitory vaginal estrus (Selye and Friedman, '40), but the stimulation of uterine growth is much greater in the intact than in the spayed animal (Korenchevsky and Hall, '37). This suggests that the normal ovary may also transform certain steroids into more folliculoid substances or sensitize the accessory sex organs to these compounds.

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[1] Working under a grant from the Banting Research Foundation.

Up to the present this particular type of "gonadotropic" effect has only been observed with testoid compounds. Preliminary experiments performed in this laboratory indicated, however, that pregnenolone (Δ^5–17(α)–ethylandrostene–3(β)–ol–17^1–one), which is practically devoid of testoid activity, also exhibits such an indirect folliculoid effect. Hence it was deemed of interest to study representatives of each independent steroid hormone action (Selye, '42) for their comparative folliculoid effectiveness in hypophysectomized rats with and without ovaries.

EXPERIMENTAL

Three experiments were performed. In the first an investigation was made of the effect of testosterone in relation to the time of the cycle at which hypophysectomy was performed, since in a preliminary (unpublished) experiment, in disagreement with Noble ('39), not all of the animals showed continuously estrus vaginal spreads. The vaginal smears of thirty normal adult female rats weighing about 150 gm. were taken bidaily for 3 weeks. At the end of this time four animals with irregular cycles were eliminated and the remaining twenty-six were hypophysectomized. Starting the day of the operation, the rats were injected bidaily, subcutaneously with 1 mg. of testosterone in 0.1 cc. of peanut oil for 3 weeks. Half of the twenty-two animals surviving at this time were then spayed. The extirpated ovaries were fixed in "Suza", weighed and sectioned. Treatment of both groups was continued with the dose increased to 10 mg. daily. Vaginal smears were taken bidaily throughout. At the end of 10 days the animals were sacrificed and the ovaries, uterus, vagina, adrenals, thymus and preputial glands were removed, fixed in "Suza" and weighed according to our previously described technic (Clarke and Selye, '43). The site of the pituitary was carefully examined for any residual hypophyseal tissue and one doubtful case was eliminated. Ovaries, uterus, vagina and mammary glands were sectioned and stained with hematoxylin-eosin.

In agreement with our preliminary experiment, only about two thirds of the animals showed estrous vaginal smears after hypophysectomy. There was no correlation between the phase of the cycle at which the operation was performed and the occurrence of vaginal cornification.

In no case did the keratinization continue for longer than 5 days and usually it did not last more than 2. After the initial estrous response the smears of all the animals contained large numbers of mucous cells and leukocytes, while a few of them showed irregular short periods of cornification of 24 hours or less. After ovariectomy and the increase in the dose of testosterone, the smears became more and more leukocytic, while in the not-spayed animals there occurred a second short period of transitory estrus followed again by the sporadic appearance of cornified spreads.

At autopsy the ovaries were atrophic and differed neither histologically nor in weight from those of untreated hypophysectomized rats. The adrenal and thymus glands were markedly and equally atrophic in both groups. The average weight of the adrenals was 14 mg. (range 9–20 mg.), while that of the thymus was 23 mg. (range 12–50 mg.). The uteri of the not-spayed animals weighed only slightly more than those of the spayed (1027 mg. as compared with 953 mg.). There was no significant difference between the weights of either the vaginae or the preputial glands of these two groups.

On the other hand, histologically the vaginal epithelium of the spayed group was less mucified than that of the not-spayed. In three animals of the latter group — but in none of the former — this epithelium was found to consist of cornified or stratified squamous cells. That testosterone is more active as a folliculoid in the not-spayed animals was also shown by the fact that the height of the uterine epithelium was 118 μ ± 13 [2] in the spayed, as compared to 180 μ ± 22 in the not-spayed. These means are significantly different from each other.

The mammary glands in both groups showed marked signs of stimulation in disagreement with the observations of Noble ('39) and the generally accepted view that the pituitary mediates the mammotropic effect of steroids. Figure 1 represents the typical atrophic mammary gland of a hypophysectomized female rat, while figure 2 shows the marked stimulation of the breast tissue which was seen in all testosterone treated animals. The ducts are enlarged, the acini appear to have grown somewhat and both are distended with secretion. There is an increase in the fibro-muscular tissue surrounding the ducts. The amount of this tissue enveloping blood vessels, nerves and even lymph channels was also greatly augmented. The degree of stimulation seen here exceeds that recently reported by Leonard ('43) with testosterone or estradiol and resembles much more closely the cystic-glandular development elicited by Astwood, Geschickter and Rausch ('37) and by Herold and Effkemann ('36) in normal male rats or in castrates of both sexes with estrone or testosterone. The extent of the stimulation is by no means comparable to that observed when the pituitary is intact, nevertheless, some development and secretion does occur in the absence of this gland providing that sufficiently large doses are administered. The failure to obtain detectable mammary stimulation with testosterone (McEuen, Selye and Collip, '37) in hypophysectomized rats, was probably due to the fact that the dosages were not sufficiently high to elicit this effect in the absence of the sensitizing hypophyseal stimuli.

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[2] Standard error $= \sqrt{\frac{\mathrm{E}d^2}{n-1}} \div \sqrt{n}$ where d is the difference between the observed value and the mean and n the number of observed values. A difference between two means is considered significant when it is more than twice the square root of the sum of the squares of the standard errors of the means.

Since hypophysectomy appeared to unmask the folliculoid activity of testosterone, it appeared of interest to investigate whether other natural or synthetic steroids would behave in a similar manner. Twenty to twenty-four female rats weighing 150–165 gm. were divided into two groups. The first group was spayed and 4 days later both groups were hypophysectomized. Subcutaneous injections of steroids suspended or dissolved in peanut oil were commenced on the day of hypophysectomy and continued bidaily for 10 days. Vaginal smears were taken daily and the animals were sacrificed 16–18 hours after the last injection. The thymus, ovaries, uterus, vagina and mammary glands were removed and fixed for 24 hours in "Suza", then transferred to 10% formalin. The first four organs were carefully dissected and weighed and the last four sectioned and stained. Thus we examined the effects

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Fig. 1 Mammary gland of female rat 10 days after hypophysectomy.

Fig. 2 Mammary gland of female rat 31 days after hypophysectomy, treated with testosterone 2 mg. daily for 21 days and 10 mg. daily for 10 days. Cyst-like dilatation and fibrosis of duct walls.

Fig. 3 Ovary of rat 10 days after hypophysectomy.

Fig. 4 Ovary of rat 10 days after hypophysectomy, injected with Δ^5–pregnenolone 2 mg. daily for 10 days. No change in structure.

Fig. 5 Uterine epithelium of rat hypophysectomized and spayed at the same time and treated with 2 mg. androstenedione daily for 10 days. Epithelium atrophic.

Fig. 6 Vaginal epithelium of same animal as shown in figure 5. Epithelium atrophic.

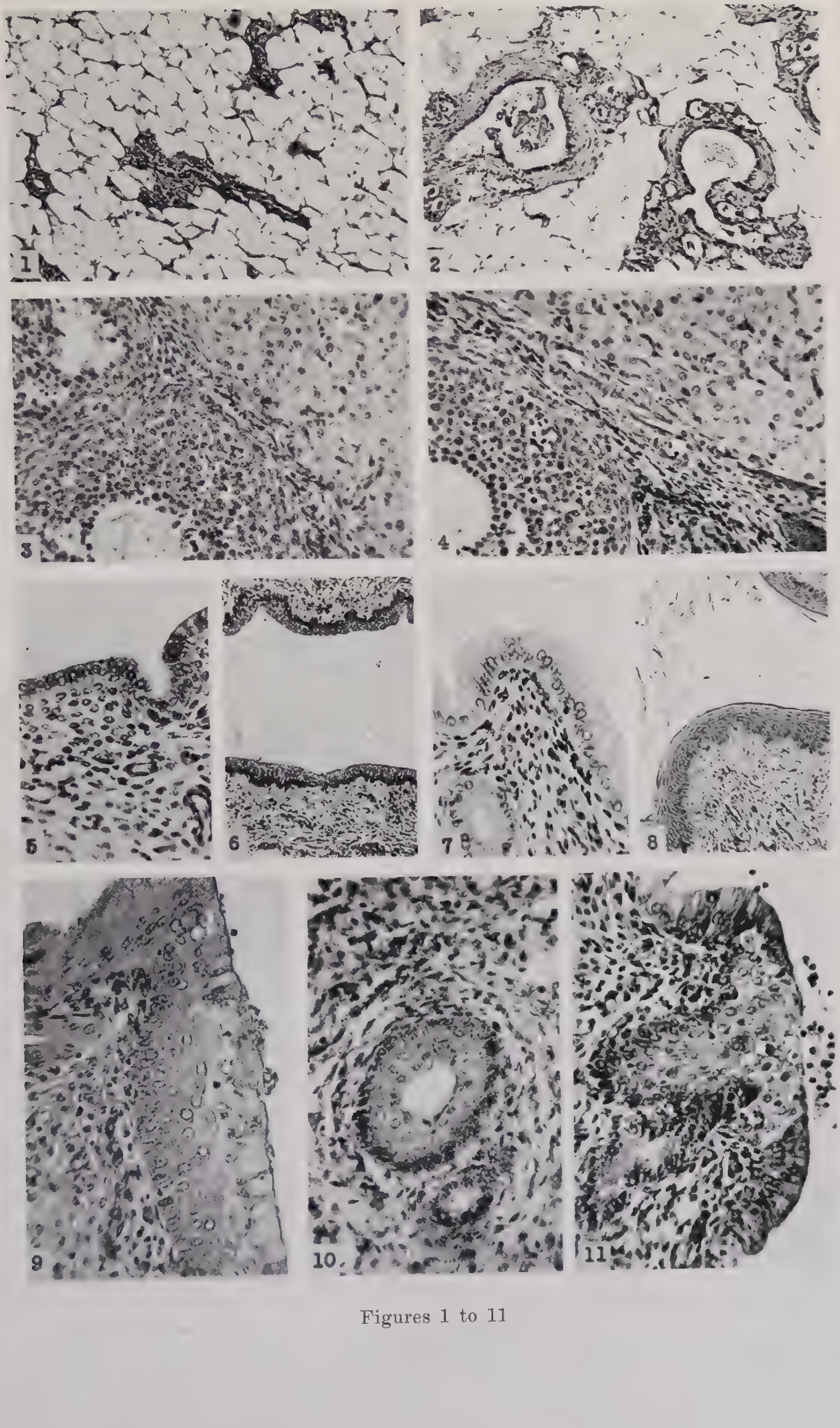
Fig. 7 Uterine epithelium of rat hypophysectomized and treated with 2 mg. androstenedione daily for 10 days. Epithelium high columnar.

Fig. 8 Vaginal epithelium of same animal as shown in figure 7. Complete cornification.

Fig. 9 Stratified squamous cell metaplasia of uterine epithelium of hypophysectomized rat treated with 2 mg. testosterone daily for 21 days and 10 mg. daily for an additional 10 days.

Fig. 10 Metaplasia of the epithelium of a deep uterine gland; note the normal adjacent gland; rat hypophysectomized and treated with Δ^5–pregnenolone for 10 days.

Fig. 11 Glandular cell metaplasia of uterine epithelium of hypophysectomized rat treated with 2 mg. testosterone daily for 10 days.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Figures 1 to 11

of estradiol, progesterone, desoxycorticosterone acetate, pregnenolone, androstenedione, and testosterone at the various daily dose levels indicated in table 1.

It will be noted that ovariectomy was performed 4 days before hypophysectomy in this experiment so that the sensitivity of the tissues of the spayed animals might have been slightly impaired. To investigate whether this would account for the marked differences seen between the spayed and the not-spayed groups, several of the steroids were tested again in an additional experiment in which both groups were hypophysectomized and one ovariectomized simultaneously. In all other respects the procedures in the two series were identical although the doses administered varied in most cases as shown in table 1. The first column of this table indicates the number of the experiment and the second that of the group. In all cases rats of group 1 were spayed, while those of group 2 were not. The steroids used are described by their popular names (in block letters) and by their systematic chemical names according to the recently proposed uniform system of nomenclature (Selye, '43). The melting points of the samples employed are given to facilitate the identification of the compounds and to give a rough estimate of their degree of purity. The dose is expressed as the total amount given daily. The number of animals indicated in each group is the number which lived through the experiment and whose pituitary was known to be completely removed by careful examination at autopsy. The average body weights at this time are also given. The rest of the table is concerned with the various hormonal effects observed. Each figure represents the average within the group and wherever possible standard errors are indicated (brackets). Vaginal smears, although taken daily, are only listed for the first and last days. Cornified spreads are designated as +, while those consisting almost entirely of leukocytes are designated as —. The intermediate state in which the smear contains a mixture of mucous, stratified squamous cells and leukocytes is indicated by ±. The histological structure of the vaginal epithelium at autopsy was recorded by the following abbreviations. A = anestrus, S = slight stratification, S_2 = moderate stratification, S_3 = marked stratification. "C" is used to indicate cornification and "M", mucification, the degrees of these transformations being recorded in the same manner as in the case of stratification. The extent of mammary gland stimulation is expressed in a scale of 0–2 in the next column. 0 indicates no stimulation and an appearance of the mammary gland similar to that pictured in figure 1. 2 represents the same type of cystic development as described above and as shown in figure 2, while 1 indicates an intermediate degree of stimulation of

TABLE 1

*The effect of steroids in spayed and not-spayed hypophysectomized rats.**

EXPERIMENT NO.	GROUP NO.	STEROID	DAILY DOSE	NO. OF ANIMALS	FINAL BODY WEIGHT (G.)	VAGINAL Smear Initial	VAGINAL Smear Final	VAGINAL Histology	MAMMARY GL. STIMULATION	HISTOLOGY OF UTERINE EPITHELIUM (µ)	WEIGHT OF UTERUS (MG.)	WEIGHT OF OVARIES (MG.)
1	1	None peanut oil	0.2 cc	7	142 (2.0)	±	—	A	0	60 (2.6)	**107** (2.5)	
1	2		0.2 cc.	9	152 (2.0)	±	—	A	0	55 (3.2)	140 (7.3)	26 (1.9)
1	1	$\Delta^{1,3,5}$–estratriene–3, 17(α)–diol α–ESTRADIOL M.P. 176–177°	1 γ	5	128 (3.1)	+	+	S_2C_2	1	131 (19)	**247** (20)	
1	2		1 γ	6	136 (4.1)	+	+	S_2C_2	1	175 (14)	337 (15)	28 (2.4)
2	1		0.5 γ	3	136 (3.2)	±	±	S_1C_1	**	279 (18)	425 (50)	
2	2		0.5 γ	6	140 (4.1)	+	±	S_2C_2	**	235 (18)	645 (125)	26 (4.0)
1	1	17(α)–[1–ketoethyl–Δ^4–androstene–3–one PROGESTERONE M.P. 128°	2 mg.	8	143 (3.5)	—	—	A	0	91 (9.5)	**171** (12)	
1	2		2 mg.	8	145 (3.6)	±	—	S_1M_1	0	99 (6.8)	288 (10)	24 (1.5)
2	1		1 mg.	6	132 (13)	±	—	S_1M_1	0	118 (5.0)	150 (5.0)	
2	2		1 mg.	4	141 (12)	±	—	S_1M_1	0	91 (14)	185 (29)	**
1	1	17(α)–[1–keto–2–hydroxyethyl]–Δ^4–androstene–3–one acetate DESOXYCORTICOSTERONE ACETATE M.P. 152°	2 mg.	8	148 (2.9)	—	—	A	0	**81** (6.2)	**95** (5.0)	
1	2		2 mg.	6	149 (3.9)	±	—	A	0	110 (6.8)	144 (9.1)	27 (1.3)
1	1		10 mg.	9	133 (3.6)	—	—	A	0	116 (6.1)	**141** (8.7)	
1	2		10 mg.	10	142 (3.4)	±	—	A	0	**98** (5.0)	195 (12)	25 (1.0)
1	1	17(α)–[1-ketoethyl–Δ^5 androstene–3(β)–ol Δ^5–PREGNENOLONE M.P. 188°	2 mg.	6	145 (4.1)	—		A	0	80 (4.6)	**103** (9.6)	
1	2		2 mg.	5	146 (3.0)	±	+	S_2C_2	0	125 (12)	234 (33)	25 (2.6)
1	1	Δ^4–androstene–3, 17–dione ANDROSTENEDIONE M.P. 169°	2 mg.	5	144 (4.1)	—	—	M_1	2	**74** (4.1)	**217** (26)	
1	2		2 mg.	9	144 (1.8)	±	+	S_2M_2 or C_2	1	251 (21)	406 (22)	26 (3.1)
2	1		2 mg.	7	148 (2.3)	—	—	S_1M_1	2	**84** (6.5)	**286** (29)	
2	2		2 mg.	4	139 (6.5)	±	+	S_3M_3 or C_2	2	206 (18)	741 (287) 429 (39)	25 (1.9)
1	1	Δ^4–androstene–3–one–17(α)–ol TESTOSTERONE M.P. 154°	2 mg.	6	147 (2.0)	—	—	S_2M_1	2	**81** (4.2)	**	
1	2		2 mg.	5	146 (2.0)	±	±	S_2M_3 or C_1	2	145 (15)	407 (40)	24 (2.0)
2	1		2 mg.	9	156 (5.6)	±	—	S_1M_1	2	91 (6.4)	403 (27)	

* For explanations of abbreviations see text.

** Not observed.

the same type. The height of the uterine epithelium in μ and the weights of the uteri and ovaries in mg. are given for each group in the last three columns.

In no case did treatment with these various steroids influence the weight of the ovaries. Histologically there was no difference in the size of the corpora lutea or primary follicles. The various ovarian cells of the treated animals differed in no way from those of the controls as indicated in figures 3 and 4. In the peanut oil treated control animals the height of the uterine epithelium and the vaginal and mammary gland atrophy were not significantly different in the two groups. The uteri of the spayed animals were, however, significantly lighter than those of the not-spayed. This difference in weight may be due in part to the fact that the former were ovariectomized 4 days before hypophysectomy. Yet this circumstance could not explain the observed difference in the uterine weights of the animals in experiment 2. Thus it does not account for the differences seen between the weights of the uteri of spayed and not-spayed androstenedione treated rats. The difference between the weights of this organ in animals spayed 4 days before and on the day of hypophysectomy is not significant, while in both experiments the not-spayed animals had significantly heavier uteri. (In the second experiment the difference was significant if the largest — apparently abnormal — uterus, weighing 1571 mg., were excluded from the calculation. In the table the first mean includes this observation and the second excludes it.) Representative sections of the uterine and vaginal epithelia are shown in figures 5 to 8. With progesterone, on the other hand, a marked metrotropic effect is seen only in experiment 1 at a 2 mg. dose level. The difference at the 1 mg. dose level tested according to the procedure of experiment 2 is not significant. The probability that in this case the difference is due to variations in tissue sensitivity is supported by the identical vaginal responses in the second experiment and by the non-significant differences between the uterine epithelial heights in both. Similarly with desoxycorticosterone acetate the differences between the uterine weights of the spayed and not-spayed groups is perhaps due to this factor. With testosterone the dose was probably too large and maximum growth occurred in both groups. That this substance was more folliculoid in the not-spayed animals is indicated by the markedly significant differences in vaginal responses and in the heights of the uterine epithelia. Pregnenolone was also more folliculoid in the not-spayed than in the spayed rat as shown by the marked difference in uterine weights, epithelial heights and vaginal smears and histology at autopsy. Finally, estradiol appears to be more folliculoid in the not-spayed than in the spayed rat according to differences in uterine weight and vaginal development (experiment 2) but not by uterine epithelial height. It would be of interest to investigate this point further since if this substance were more active in the presence of the ovary it would suggest that the true ovarian hormone is not estradiol. The present data is merely suggestive. It is interesting that with this compound the lower dose is much more effective in increasing the weight of the uterus than is the higher dose.

In these experiments four instances of uterine metaplasia were observed. Previously, metaplasia of the uterine epithelium has been seen only with excessive and prolonged administration of active estratrienes. Metaplasia with testosterone was seen first in a not-spayed hypophysectomized rat in the first experiment reported above in which large amounts were given for a considerable period of time (fig. 9). In the short-term experiments one case appeared in one of the not-spayed groups of animals treated with testosterone, androstenedione and pregnenolone but not in any of the rats given estradiol or any of the other compounds. The portion of the uterus taken for section was about 5 mm. above the angle of the two horns. Only about eight to ten consecutive sections were cut hence possibly metaplasia occurred more commonly than these observations would suggest. Since serial sections were examined errors due to tangential sectioning could be avoided. The metaplasia was not extensive but patchy. It occurred in the deep glands as seen in figure 10 or in the epithelium of the lumen. The cell-types were either stratified squamous, as seen in figure 7, or resembled mucified vaginal cells as seen in figure 11. In no case did metaplasia occur in any of the spayed animals. Thus the data would suggest that the ovary is able to transform testosterone, androstenedione and pregnenolone into some folliculoid substance which actively promotes uterine epithelial metaplasia.

SUMMARY AND CONCLUSIONS

1. Testosterone, pregnenolone and androstenedione were found to be more folliculoid in the not-spayed than in the spayed hypophysectomized rat. On the other hand, the folliculoid potency of progesterone, desoxycorticosterone acetate and estradiol was only doubtfully increased.

2. Cystic mammary development was observed in hypophysectomized rats with androstenedione, testosterone and, to a much smaller extent, with estradiol.

3. Four cases of uterine metaplasia were observed in not-spayed, hypophysectomized rats with testosterone, androstenedione and pregnenolone.

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LOCALIZATION OF MAXIMUM CELL DIVISION IN EPIDERMIS[1]

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There is difference of opinion as to the layer in the epidermis in which cell division is maximal. Pinkus ('27) believes that it is the basal layer and goes so far as to state that mitosis in this layer is the only normal mode of epidermal replacement. Thuringer ('24 and '28), on the other hand, has found more mitoses in the spinous than in the basal layer. He has, moreover, presented evidence ('39) that on stimulation these two strata respond by increase in mitosis independently. This may afford some clue to the origin and sharply contrasting properties of basal and spinous (squamous) cell carcinoma. For our own studies in Barnard Hospital the whole problem of cellular relations in the epidermis needs clarification. Among the changes in epidermis under the influence of methylcholanthrene that are being investigated, alterations in mitotic frequency are important. These have been studied in whole mounts of epidermis, viewed vertically, by Cooper and Reller ('42) and by Reller and Cooper ('44). The whole mount method has great advantages over the examination of sections in the making of total counts involving all the layers. But sections are essential for differential counts of mitoses in the several layers. The trouble has been that mitoses are so rare that great numbers of nuclei have to be examined before a sufficient number of mitoses are located to give an accurate measure of their frequencies. For instance in the epidermis of the foot pads of cats, Thuringer ('39) found only 500 mitoses in 210,765 cells, a ratio of 1:421; while Cooper and Franklin ('40) found in epidermis of the ears of mice a maximum of fifty-nine mitoses per 15,000 nuclei, a ratio of 1:250. The object of this study was to test the applicability of a method of concentrating the mitoses, which has proved useful in other tissue, and possibly with its help to obtain data on normal epidermis which would serve as a basis for later studies on epidermal carcinogenesis.

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MATERIAL AND METHODS

Mice of new Buffalo strain were chosen as material because of their use by others in this hospital concentrating their attention on epidermal carcinogens. It was not feasible to work with normal epidermis of the ears or back because of its extreme thinness. Preliminary observations showed that the regionally hyperplastic epidermis of the hind foot pads was satisfactory. Though rather more cornified, it is of approximately the same thickness and exhibits about the same layering as the epidermis of the back made hyperplastic with methylcholanthrene in which malignant foci are likely to appear. It was decided to limit the observations to young actively growing mice because in them one would expect the layer of maximum cell division to be more sharply marked than in adults.

The method of concentrating mitoses was the same as that employed with conspicuous success by Allen, Smith and Gardner ('37) in localizing the growth effect of theelin in the female genital tract. It depends on the action of colchicine in allowing mitosis to begin and in holding up completion of the process so that they accumulate. In a preliminary experiment the optimum dosage was determined.

For the main experiment a litter of six 10-day-old mice was selected. At 8 A.M., 12 noon, 4 P.M., 8 P.M. and midnight one mouse was injected subcutaneously with 0.25 cc. of 1: 10,000 aq. colchicine. At 4 A.M. the next morning all were sacrificed together with one untreated mouse to serve as control. The hind footpads were excised and fixed in Bouin's fluid. After a few minutes they were trimmed with a sharp razor blade, imbedded in paraffin in the usual way, sectioned at 8 μ and stained with hematoxylin and eosin.

OBSERVATIONS

All the counts were made by one of us (H. C. T. Jr.) with the aid of a mechanical stage. The nuclei not in mitosis were enumerated by an ordinary mechanical counter, while the mitoses were recorded by hand on a sheet of paper (table 1).

Thus, in the normal untreated control epidermis 1826 nuclei of basal cells (actually in touch proximally with the basement membrane) were counted. Among these, forty-six were in mitosis so that the mitotic ratio was 1: 39.7.

In the remaining epidermis, consisting of cells distal to the basal ones, it was not possible sharply to delimit the distal margin of the spinous layer. Consequently all the non-basal nuclei were included.

TABLE 1

Mitotic ratio in basal layer and rest of epidermis.

TIME AFTER COLCHICINE INJECTION	BASAL LAYER Nuclei in mitosis	BASAL LAYER Total nuclei	BASAL LAYER Ratio	REST OF EPIDERMIS Nuclei in mitosis	REST OF EPIDERMIS Other nuclei	REST OF EPIDERMIS Ratio
Control	46	1826	1: 39.7	121	4400	1: 37.1
4 hrs. (midnight to 4 A.M.)	12	524	1: 43.6	40	1300	1:32.5
8 hrs. 8 P.M.– 4 A.M.)	43	952	1: 22.1	158	2952	1: 18.6
12 hrs. (4 P.M.– 4 A.M.)	6	792	1: 132	54	1449	1: 26.7
16 hrs. (Noon– 4 A.M.)	6	584	1: 97.3	54	1276	1: 23.6
20 hrs. (8 A.M.– 4 A.M.)	6	625	1: 104	42	1622	1: 38.6
Totals	119	5303	1: 44.5	469	12999	1: 27.7

These amounted in the normal epidermis to 4400 among which 121 were in mitosis so that the ratio was 1: 37.1.

Ratios for the specimens at various times after the injection of colchicine follow in the table data on the normal epidermis. It is clear that throughout the series the ratios are higher for the suprabasal nuclei than for the basal ones. The highest ratios were found in both 8 hours after colchicine injection at which time its action is generally considered to be maximal.

When the mitoses were counted in the suprabasal layers a record was kept of whether they were located in the inner, middle or outer third of this portion of the epidermal sheet. For the sake of clearness these data are presented in a second table (table 2). They show that, in all the treated epidermises, more mitoses were always observed in the middle than in either the proximal or the distal thirds of this suprabasal sheet.

The cellular population of this middle third obviously differs from that of the other two. The cells are larger and possess more pronounced spines than in the proximal third. They are also larger and do not show the granulation and cornification evident in many cells of the distal third.

The fact that in the control epidermis more mitoses were found in the proximal third than in the middle third is important because it shows that level of maximum mitosis is not fixed.

TABLE 2

Incidence of mitoses in parts of suprabasal epidermis.

TIME AFTER COLCHICINE INJECTION	PROXIMAL ⅓	MIDDLE ⅓	DISTAL ⅓	TOTAL
Control	77	33	11	4400
4 hrs.	8	24	8	1300
8 hrs.	38	103	17	2952
12 hrs.	12	33	9	1449
16 hrs.	14	34	6	1276
20 hrs.	12	27	3	1622

DISCUSSION

Cautious interpretation of these results is indicated, because there are several factors that may possibly have influenced them.

Cooper and Franklin ('40) have discovered that a diurnal rhythm exists in frequency of mitosis in epidermis of the ears of mice with gradual increase to a maximum at 10 A.M. and decrease to a minimum at 10 P.M. Consequently it is likely that the frequency of cells entering into mitosis in the epidermis of footpads will not have been uniform throughout the period of our experiment extending from 8 A.M. to 4 A.M. It is doubtful, however, whether this alone would materially influence the location of maximum cell division. Only further work will show whether during the rhythm there is a change in level of maximum mitosis. This is not suggested, but is only held as a possibility. Even so the magnitude of the shift might not be sufficient temporarily to govern the level in our specimens.

In another paper of this series by Paletta and Cowdry ('42) mention has been made of the possibility, noted occasionally by investigators working with other tissue, that colchicine may to some extent either increase or decrease the number of nuclei entering into mitosis. Unless this influence, one way or the other, acted differently on the several layers it might be expected to bring about an increase or a decrease in the maximum frequency of mitosis, wherever that is located, without causing any shift in location. Until evidence is presented of the existence of a mitosis stimulating or inhibiting influence of colchicine acting differentially on the layers of mouse epidermis this possibly disturbing factor, though kept in mind, need not be further discussed.

It is also conceivable that the summation effect of the drug on mitoses will be greater in the basal layer of cells, which receives it first and most directly from the tissue fluid beneath, than in the more distant suprabasal cells. If this is so, and the cells were otherwise equal in mitotic activity, one would look for a higher incidence of arrested mitoses in the basal than in the suprabasal cells. This gives added significance to the fact that the highest incidence in the colchicine treated epidermises was not only in the suprabasal epidermis but in its middle third; for, on this hypothesis, a normally higher incidence of mitosis in this third might be somewhat reduced relatively by greater summation of mitoses in the basal cells.

There is a second consideration which strengthens the conclusion that, in the treated specimens examined, the highest incidence of mitosis was actually in the middle epidermis. In dividing this into three parts, based on thickness of tissue distal to the basal layer, fewer cells were included in the middle third than in either of the other two; because in it the cells were larger than in the proximal and distal thirds. If the frequency of mitosis were the same one would anticipate the finding of fewer mitoses in the middle third than in the proximal third. For a small number of cells to have exhibited more mitoses than a larger number means that the frequency was relatively even higher in the middle than the figures indicate.

Observations by Thuringer ('39) are of interest in connection with ours, because he used the same kind of epidermis (palmar and plantar pads) though not of mice but of cats. He observed the distribution of 500 mitoses, expressed in percentages, to be: basal layer, 44.5; proximal third spinosum, 54.5; middle third spinosum, 1; and outer third spinosum, 0. From this he concluded that "the lower one third of the stratum spinosum was more active than the stratum basale" which is obviously justified from his data. His published figures do not, however, permit calculation of relative mitotic frequencies in these layers; because, while the number of mitoses in each are given, the number of cells is not. If the number of cells examined were equal in both, then it could be said that the mitotic frequency was slightly higher in the proximal third of the spinosum than in the basal layer but if it were significantly greater in the former this would not be the case.

On the whole it seems that our observations place the stratum of maximum frequency of mitosis in the tissues we studied more distally than is likely to have been the case in Thuringer's "resting" material; for, it must be remembered that the suprabasal epidermis, which we artificially divided into three parts, included some tissue distal to the true spinous layer so that our proximal and middle thirds of the suprabasal epidermis included more than two thirds of the spinous layer.

On electrical stimulation Thuringer discovered an impressive shift in the location of the greatest number of mitoses. Those in the lower third of the spinosum exceeded those in the stratum basale by as much as 653 to 100. In view of this finding, and of our observation of maximum mitoses in the proximal third of the suprabasal epidermis of the untreated specimen whereas the maximum was always in the middle third of the treated specimens (table 2), it would be unwise to assume that the level of maximum incidence of mitoses in epidermis is fixed. On the contrary this level can probably rise and fall in different physiological and pathological conditions depending on many factors including increase and decrease in the amount and character of fluids seeping up into the epidermis from the tissue fluid beneath.

The discovery in our material and in Thuringer's that the maximum frequency of mitosis was centered somewhere in the spinous layer is not to be interpreted as indicating that cells are mainly produced by multiplication of preexisting ones in this layer and are displaced in both directions.

All the evidence points to removal of worn out cells from the distal surface of the epidermis. Signs of death of basal cells are few and far between. Consequently there is as yet no valid reason to recast the old idea that the epidermis is constructed of layers of cells shifting in a distal direction and taking origin ultimately from basal cells in contact with the basement membrane.

We can picture these basal cells as vegetative intermitotics in the sense defined by Cowdry ('42). Their individual lives are intermitotic, since they extend from one mitosis to another. They can be regarded as vegetative; since, on multiplication, some of the daughter cells remain as basal cells close to the basement membrane, vegetative and no more differentiated than their forebears. Other daughter cells are displaced distally and start out on the path of differentiation. These, by contrast, are differentiating intermitotics, whose lives extend likewise from one mitosis to another. Each one begins its individual life a little further differentiated than its predecessor so that differentiation is in a series of ascending cellular steps until cells are produced, which are handicapped by increasing distance from the life giving tissue fluids, and which are postmitotics in the sense that their individual lives end in death, not in another mitosis.

The finding of maximum mitotic frequency in the spinous cells of the middle third of the treated suprabasal epidermis in our specimens simply means that these particular differentiating intermitotics are multiplying more rapidly than the other cells just as primary spermatocytes of the testicle can and often do divide more frequently than the spermatogonia, the order of replacement being the same in the testicle as in the epidermis.

SUMMARY

In the epidermis of the hind foot pads of an untreated 10-day-old new Buffalo mouse the ratio of mitoses to nondividing nuclei was 1:39.7 in the basal layer and 1:37.1 in all the suprabasal layers taken together. In the litter mates, each of which received 0.25 cc. 1:10,000 aq. colchicine subcutaneously, the ratios increased to a maximum of 1:22.1 and 1:18.6, both 8 hours after the injection. In the untreated animal the site of maximum mitotic frequency was in the spinous cells of the proximal third of the suprabasal epidermis, while in all of the treated ones it was located in those of the middle third. The possibility is suggested that the level of maximum mitoses in the epidermis is not fixed but subject to change in different physiological and pathological conditions.

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BOOK REVIEW

THE VERTEBRATE EYE AND ITS ADAPTIVE RADIATION. By GORDON LYNN WALLS. Published by The Cranbrook Press, Bloomfield Hills, Michigan, 1943, 14 + 785 pp., 197 illustrations. Price $6.50.

The title of this book is not wholly self-explanatory. It appropriates the term "adaptive radiation," which Prof. H. F. Osborn coined to describe a way of diversification among animal groups, and uses it to indicate "what the vertebrate eye has done in modifying its pattern to fit itself for the many different kinds of performance demanded of it by its adaptively-radiated owners [that have] poured themselves into a number of environmental molds which were made available to them more or less simultaneously." The book is divided into three sections; although these are not unrelated, each might almost qualify as a separate book. Part I, captioned "Basic," covers 139 pages. Its aim is to furnish the uninformed with elementary information concerning the development, structure and function of the eye. This is in accordance with the author's plan to make his volume intelligible to many different kinds of people who need have no more scientific knowledge as a prerequisite than could be obtained from an elementary course in biology. Although this section enlarges the size of the total volume considerably, not all of its content is without interest to the more advanced student. Here and there will be found items that will refresh or extend his ideas in a rewarding manner. Examples are "Types of visual cells," "The duplicity theory" and "Photochemistry of color vision." Of arresting interest is the author's elaboration of Studička's theory that the visual cell is a specialized ependymal cell.

Part II, entitled "Ecologic," (309 pages) is the body, and soul, of the book. In concept and execution it reveals the author at his best. Actually it deals with the numerous adaptations, structural and functional, that the eye has undergone in relation to different modes of life and environmental settings. Here will be found a wealth of facts that have been made to live and take on significance through the multiple correlations of structure, function, animal conduct and environment. For how much of this synthesis the author deserves exclusive credit, the reviewer cannot say. The absence of documentation makes such an evaluation difficult. Although some modest attempts in this direction have been made before, as by Wunder, and although many publications include fragmentary correlations of specific items, no one has ever attempted in so ambitious a manner to tie all the relevant facts into a harmonious whole. It is the considered opinion of this reviewer that Dr. Walls should be accorded all the credit that goes to any scientific pioneer who has the vision to advance into an unmapped region, the hardihood to explore it, and the critical ability to so organize his explorational data that the report will serve as a reliable guide to colleagues in the same field of scientific endeavor. Because of the diversity of the subjects treated, and the ramifications of each, it is impracticable to do more than enumerate the chief topics dealt with in this section.

With respect to the arythmic (nocturnal-diurnal) eye are discussed photomechanical changes in the retina and their utilities, pupillary mobility (which in mammals the author argues has replaced the advantages furnished by the older and slower photomechanical-change mechanism), and the duplex retina, good for both day- and night vision. The author challenges the generally accepted belief that the rod is the primitive visual element and the cone an improved derivative; on the contrary, he maintains that "the first rods in the world were produced by the transmutation of cones, and that the process has been occasionally repeated, wherever needed, ever since vertebrates came on land." Less often, rods are said to have transmuted into cones.

Diurnal habits have been accompanied by an increase in visual acuity at the expense of sensitivity. The roles that are played in diurnal vision by an increased image-size, by a predominant or pure cone-retina, and by an area centralis are discussed. In treating the area centralis the author develops his theory that the fovea is not a pit that finds significance through the advantages accruing from mere thinness; rather, it is a refractive device by which an image is expanded to larger size and thus comes to be analyzed by more unit receptors. Another means of promoting visual acuity is by the introduction of intraocular color filters; in the exploration of this field the author shows how the yellow lens and macula of man serve to absorb the short waves that contribute most to the production of chromatic aberration.

Nocturnal habits are correlated with a rod-retina of high sensitivity but, usually, of low resolving power. Various adaptations have developed that serve to enhance night vision. These include: relatively large eyes and even absolutely large ones; altered eye-ball shapes; a widely-opening pupil and a proportionately large, spherical lens; dislocation of the lens to a position farther back from the cornea, resulting in a smaller but brighter image. The slit pupil of nocturnal animals is really a protective device that enables a pupil to close much more completely than does an ordinary sphincter; with it an animal with a rod-rich retina of the nocturnal type can also venture out in the sunlight. The chorioidal and retinal tapeta, and their roles as reflectors, are treated with thoroughness.

The discussions under adaptations to space and motion include such topics as: the devices that accomplish accommodation; substitutes for accommodation; visual angles and fields; the binocular field, and devices for enlarging it; eye movements and the fovea; depth- and solidity perception; movement perception. Under adaptations to media and substrates are considered: aquatic vision; aerial vision; combined air- and water vision; protective "spectacles." Under adaptations to photic quality are treated: color vision; dermal color changes; eye coloration.

Because of the ecological viewpoint maintained in this section, certain fields of knowledge find no place in it. Hence the book does not attempt to treat with such topics as: ocular biochemistry; retinal photoelectrics, physiological optics, etc.

Part III, "Synoptic," (135 pages) traces the structure of the eye, group by group, from the lowest vertebrates to the highest. Each group is treated under "the eye as a whole" and "the retina." Within these descriptions will be found those structural features which are important to the eye as an organ, even though they are not known to be related to the conduct of an animal in its natural environment. The emphasis is placed "on the morphology of the eye, the evolution of that morphology, and the bearing of it upon the problems of vertebrate phylogeny." Thus the viewpoint is quite different from that which dominates Part II. Probably few will find it equalling the interest of that section, yet there are fascinating topics to be found; such is the incomparable analysis of the pecten of birds. Nevertheless, Part III constitutes an important, systematic encyclopedia that will be of great practical service to all, since everyone needs, from time to time, an authoritative reference source on these matters.

There are 197 figures, a bibliography of key titles, and an index which is also a glossary for taxonomic terms.

Several general criticisms might be leveled at this book and it is only fair to enumerate some of them. The author commonly states his personal beliefs and opinions in terms of inflexible dogmatism; also many things that may have or presumably did happen in a certain way are unqualifiedly said to have so happened. There is constant use of teleological expression in explaining why adaptations have come to be what they are. Lower animals are credited with visual acts in terms that imply the acquistion of the equivalent of a human brain. To be sure, the author has explained that some of these styles have been adopted deliberately and purely as economy devices, but it is doubtful whether most readers will find and read these explanations.

There can be no question that "The Vertebrate Eye" is an important book. This would be true if for no other reason than that it is a unique synthesis of material that has long needed this kind of organization. To the specialist in this field it is a book to be kept always within reach. To the general scientific reader it is a volume written in such an informative and sprightly style that much of it is definitely "good reading." Both author and publisher are deserving of the highest commendation for their production of this work. It will be a standard reference wherever and whenever information on the eye as a biological mechanism is needed.

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BOOK REVIEW

MEDICAL RADIOGRAPHIC TECHNIQUE. By Technical Service Department of General Electric X-Ray Corporation under the editorial supervision of Glenn W. Files, Director. Cloth. Price, $6.00. Pp. 365. Springfield: Charles C. Thomas, Publisher.

The expansion of the field of x-ray technique and the increasing demands for technicians should make a new book written for technicians by technicians most welcome. In this volume the section on the physics of x-ray technique is well done, and with some attention on the part of a moderately well grounded individual, it should permit a good understanding of the principles underlying the use of x-rays for radiography. The material on normal anatomy is obviously brief and rudimentary but should offer a guide to the limitations of teaching of anatomy to technicians. The skeletal anatomy is well presented with good drawings which are fairly well labeled. Unfortunately the authors did not see fit to follow through in the same fashion with the visceral anatomy, so that the few plates here are quite inadequate, and the anatomical demonstration is much too brief to be of sufficient value. Some of the roentgenograms of sagittal, coronal, and transverse sections of cadavers, which have been made by one of the authors working at Northwestern University, should be of considerable interest to anatomists. The standard positions for radiography are well demonstrated by photographs and diagrams, although somewhat incomplete.

It is difficult to understand why a modern text on roentgen technique would omit completely such things as bronchography, roentgenkymography, the structure and uses of the casette, radiography of the stomach upright, or some considerations of bedside radiography. There are other less serious omissions. The illustrations are well done, although the attempt of the authors to indicate the effects of different techniques by the way of fine differences in the roentgenogram are not always borne out by the reproductions.

While there are many features of this book which are highly desirable, it is somewhat disappointing. It is to be hoped that an early revision will permit changes which will fulfill the promise of the better features.

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For the second year in succession the annual meeting of the Association has been omitted because of the heavy demands placed upon transportation facilities by war necessities. Again, however, abstracts have been received and are printed herewith.

(The excellent arrangement of author cross-indexing introduced last year by Dr. Swett has been given up "for the duration" as a space-saving measure.)

The effect of nembutal anaesthesia upon the response of arterioles to epinephrin and histamine, as seen in transparent moat chambers in rabbit's ears. **Richard G. ABELL and Robert CHAMBERS**, Department of Anatomy, Medical School, University of Pennsylvania, and Laboratory of Cellular Physiology, Department of Biology, Washington Square College, New York University.

Although nembutal anaesthesia has been used extensively in studies of experimentally induced shock, direct microscopic observations of the effect of such anaesthesia upon the response of arterioles to epinephrin and histamine have not been reported.

In the present experiments the extent of arteriolar constriction produced by intravenous injections of 0.00015 mg. of epinephrin, and the degree of dilatation caused by intravenous injections of 0.5 mg. histamine were measured with a micrometer ocular in unanaesthetized rabbits, and photographed. The effects upon the same arterioles of intravenous injections of the same amounts of these substances were then measured and photographed during anaesthesia produced by intraperitoneal injections of 30–40 mg./Kg. of nembutal. The anaesthesia did not alter the responses.

In a typical experiment, injection of 0.0015 mg. of epinephrin caused an arteriole 50 microns in diameter to constrict to 25 microns. During nembutal anaesthesia the same amount of epinephrin caused the same arteriole to constrict to the same extent. Before anaesthesia, injection of 0.5 mg. histamine caused this same arteriole to dilate to 65 microns. Anaesthesia did not alter the response.

The smallest arterioles studied had control diameters of 12 microns, and the largest 75. All were newly formed and were derived from those of the subcutaneous connective tissue of the ear.

Three rabbits were used, 18 arterioles studied, and 20 injections of epinephrin and 12 of histamine made.

Direct observations on the effect of hemorrhage upon the mesenteric blood vessels and blood flow, as seen in the dog. Richard G. ABELL and Irvine H. PAGE, Department of Anatomy, Medical School, University of Pennsylvania, and the Lilly Laboratory for Clinical Research.

In these experiments a portion of the intestine and its attached mesentery were drawn from the body cavity and inclosed within a transparent celluloid chamber in each of 11 dogs. The animals were anaesthetized with 30 mg./Kg. nembutal injected intraperitoneally. Observations with the microscope at magnifications of 200 and 400 diameters showed that removal of blood in amounts sufficient to cause the pressure to fall to 30 mm. Hg. (usually 25–55 cc. per kilo) caused constriction of the arteries and veins, and marked reduction in the blood flow.

The larger arteries (0.39–1.2 mm. diameter) constricted 20 to 60%, and the smaller ones (0.024–0.065 mm.) to a like degree. The larger veins (0.1–2.1 mm.) also constricted, the degree being 12 to 43% of their control diameters.

At pressures of 30 mm. Hg. the arterial flow was seen to be slow and pulsatile, and in some instances intermittent. Stasis was frequently present in many of the capillaries and venules.

In six of the dogs no blood was returned, and all died within 1½ hours after the pressure had been reduced to 30 mm. Hg. In one of the dogs, all of the blood was returned after ½ hour at 30 mm. Hg. The arterioles relaxed to their control diameters, the blood flow once again became rapid, and the animal survived until sacrificed 3 hours later.

The effects of adrenalin injections on male newts. A. Elizabeth ADAMS and Elsie F. HUNTER, Department of Zoology, Mount Holyoke College.

The relationship between adrenal medulla and gonad was investigated by injecting adrenalin chloride (Parke, Davis & Co., 1: 1000 'adrenalin') intraperitoneally twice weekly, or more often in one group, into normal and unilaterally castrated male Triturus viridescens for 8 weeks. Total doses per newt were 1.6 cc. or 3.2 cc. Unilateral castrates had been operated 1 week or 8 weeks before injections started. Controls received 0.4% NaCl.

By the end of treatment, all experimental and control groups had lost body weight, but adrenalin-injected newts more than controls. The testes in both normal and unilateral castrates receiving adrenalin weighed much less than those in comparable control animals autopsied at the same time and than those removed at castration. In spite of this loss varying amounts of spermatogenesis occurred in all experimental and control groups. The testes of adrenalin-injected newts showed considerable degeneration.

Histological examination of the anterior pituitaries of the newts unilaterally castrated for 8 weeks and given adrenalin for the following 8 weeks revealed degenerative changes in their cells, implying that a diminished gonadotropin production might be affecting the testes. Many of the experimental data, however, suggest that a 'pathological inanition' produced by the adrenalin injections may be fundamentally responsible for the altered pituitary and testicular states, rather than a direct antagonism between adrenalin and gonadotropins or adrenalin and testicular hormone.

The extent of the carotid pressoreceptor area in the cat as indicated by its special elastic-tissue wall. William H. F. ADDISON, Department of Anatomy, University of Pennsylvania.

In the cat the internal carotid artery and the occipital artery usually arise close together in the region where the common carotid artery divides. The internal carotid artery is smaller than the occipital artery. The glomus caroticum is situated on the occipital artery close to its origin. Serial sections of the region including the above structures show that there is a small outpouching from which both the internal carotid and the occipital arteries arise, and that the special elastic structure of the vessel wall, characteristic of the carotid sinus in other species, is present in the entire outpouching and in the first part of the occipital artery as far as the glomus caroticum. Beyond the glomus the occipital artery changes to the muscular type of artery. Thus in the cat the pressoreceptor area, as indicated by its special elastic structure, is not confined to the dilated beginning of the internal carotid artery but is an area common to both the internal carotid and occipital arteries. This arrangement in the cat will be compared with that in the dog, where in addition to the well developed carotid sinus there is at the beginning of the occipital artery a small dilatation with a special elastic-tissue wall similar to that of the carotid sinus.

On the implantation of the rat egg. Roland H. ALDEN, Division of Anatomy, University of Tennessee.

In the albino rat primary implantation of the egg occurs, without exception, in the antimesometrial quadrant of the uterus. This is the portion of the uterus which the egg might be expected to contact first were gravity the governing factor in determining its position. In order to ascertain whether alteration of uterine polarity influences the implantation site, twenty-one trial experiments have been performed.

The middle third of the left uterine cornu was resected, rotated 180 degrees on its longitudinal axis, and reanastomosed. The uterine artery and its branches were left intact. Following recovery, the animals were mated and the early implantation sites subsequently studied histologically. The right cornu served as control. All successful experiments are in substantial agreement; the egg implants in the antimesometrial side of the uterus in both reversed and unreversed segments.

These findings suggest that something other than gravity induces the primary antimesometrial attachment of the egg.

The innervation of the biliary system of the rat. William F. ALEXANDER and Encil M. BRADLEY, Department of Gross Anatomy, University of Georgia, School of Medicine.

The biliary system of the rat receives its main nerve supply from the celiac ganglia. The nerves reaching the porta hepatis accompany the hepatic artery and portal vein. Extirpation of the celiac plexus removes all of the nerves passing into the liver. A few fibers remain in the porta, but their source is obscure.

Nerve strands from the vagi can be traced towards the porta hepatis, but do not pass into the liver. They course inferiorly to reach the pancreas and duodenum. Sectioning the vagi and subsequent microscopic examination reveals no loss of fibers in the porta.

The nerve fibers in the portal canals form an interconnected plexus predominantly associated with the branches of the hepatic artery. They are mostly unmyelinated fibers. A few myelinated fibers are contained within the larger nerve rami, but their origin and termination have not been determined. Nerve fibers have not been observed within the liver lobules.

Ganglia or ganglion cells have not been observed within the porta hepatis or in the liver. A few are found in the vicinity of the distal portion of the common bile duct, but due to the intimate relation of the duct to the pancreas we are unable to tell whether these are associated with the pancreas or the duct.

The similarity of neuroglia to other connective tissue. Warren ANDREW and Charles T. ASHWORTH, Deparments of Anatomy and Pathology, Southwestern Medical College, Dallas, Texas.

A study of the neuroglia fibers, especially in their relationship to the fibrous astrocytes, has been made. Portions of the nervous system of man, dog, calf, hog, and mouse were used.

The neuroglia fibers were stained by Foot's method for astrocytes. They appear in all of the species studied primarily as ''border fibers'', resting on the surface of the cytoplasm, as described by Mallory. We find no reason to call them intracellular. These fibers are much more frequently undulatory in their course than we would suppose from the literature. They definitely aid in forming investments for the blood-vessels in certain parts of the nervous system. They also form a reticulum which is well defined beneath the pia and, in a finer form, apparently occupies the major part of the central nervous system.

The species differences in the size of the neuroglia fibers are of great interest. The largest fibers are found in the dog, particularly in the white matter of the cerebral gyri. Here some fibers are relatively enormous, having a diameter greater than that of the astrocyte cell-bodies themselves. Such fibers retain their topographical relation to the cell and pass from the cell to form a fibrous, argyrophilic investment for small blood-vessels.

The neuroglia comprises a true connective tissue of cells and fibers, the latter not within the cells but as border structures.

The true relationship of fibers to cells in neuroglial tissue. (Demonstration) Warren ANDREW and Charles T. ASHWORTH, Departments of Anatomy and Pathology, Southwestern Medical College, Dallas, Texas.

Slides and photomicrographs are presented to show how neuroglia fibers are related to fibrous astrocytes and why the true relationship is not apparent in many preparations. A high degree of differentiation between the cytoplasm, best impregnated only a light grey, and the fibers, definitely black at this stage of impregnation, is required. Cajal's gold chloride-sublimate method, while showing the astrocytes very well, makes the fibers appear as parts of the cytoplasmic processes. The silver methods frequently show the entire cell, processes, and fibers, as one completely black structure in which details are not seen.

Adequate differentiation of cell cytoplasm and neuroglia fibers in the nervous system of a number of species, including man, dog, calf, hog, and mouse, shows the fibers to be ''border fibers''. Contrary to many modern descriptions, they are not intracellular structures but resemble closely the argyrophilic fibers of early connective tissue in other parts of the body.

The resemblance of these fibers to those of other connective tissue is shown also in their frequently undulatory course, in their varying size, and in their formation of investing coverings for blood-vessels. The largest neuroglia fibers demonstrated are those in the white matter of the cerebral gyri of the dog, where individual fibers frequently have a diameter as great as that of an astrocyte cell-body.

Investigation of the temporomandibular joint. J. Lawrence ANGEL, The Daniel Baugh Institute of Anatomy, Jefferson Medical College. (Introduced by George A. BENNETT.)

Observations of Dr. F. P. Lord suggested study of the path of the mandibular condyle along the articular eminence slope. On parasagittal section drawings the angle between this slope and the alveolar plane averages 34–36° among European groups ($N = 137$) and 23° among Eskimos ($N = 72$). Flatness of the Eskimo glenoid fossa has been attributed to environmental rigors, an hypothesis contradicted by the relative steepness of the Medieval Icelandic articular eminence despite ''eskimoid'' diet. With relatively flat articular eminence the Eskimo combines long face and skull base, relatively horizontal lowest external pterygoid muscle-fibers, and an edge-to-edge bite, contrasting with the European combination of opposite features and suggesting racial and functional determination of eminence slope.

Ancient Greeks show no ''eskimoid'' trend linked with flat articular eminences, though two Greek racial subtypes do diverge significantly in eminence slope. Slope steepening is significantly associated with increasing age and with loss of teeth, containing the pre-adult steepening linked with tooth eruption. Possible tendencies toward steepening with increasing teeth wear or with extreme overbite cancel out because of contrasting associations of overbites with unworn teeth and of eminence slope and teeth wear with age.

Thus heredity is the main determinant of articular eminence slope, though inherently complex mechanical factors produce great individual variety. But correlations never reveal precise causes, and further research is planned.

The human stapes, a nonconformist among bones. Barry J. ANSON, Earl W. CAULDWELL and Arthur F. REIMANN, Department of Anatomy, Northwestern University.

A study of numerous series of the ear (chiefly in Professor Bast's collection) reveals some remarkable features in stapedial development.

Ossification of the stapes spreads from a single center on the obturator surface of the base (150 mm. CR), to crura and neck (160 mm.). Periosteal bone, thus becoming continuous circularly (183 mm.), extends to the lipped margin of the base and to the medial margin of the head. Internal to this shell, cartilage is converted into endochondral bone; concurrently the obturator surface is perforated extensively for the transmission of osteogenic buds. Coalescence of these foramina result in removal of bone on almost the entire obturator surface. In the base some bone is salvaged by fusion with the persisting endochondral bone (120 mm.); endochondral bone remains internal to the articular cartilage of the head, making that portion bilaminar (245 mm.). The periosteum does not act to increase perceptibly the dimensions of the stapes; epiphyseal areas for longitudinal growth are wanting. Thus lacking the customary mechanisms for expanding and lengthening, ''adult'' dimensions are attained in midfetal life.

The crura are converted into channelled columns, the head into an excavated cylinder with bilaminar articular extremity, and the base into a thin, two-layered plate. The several parts have been excavated to expose their interiors circumferentially to the obturator foramen — at the expense of space which originally contained marrow and endochondral bone; the osseous surface thus exposed by erosion becomes covered by mucous membrane, and its adjacent tissue is converted from marrow into submucosa.

On the source of origin of the Brenner ovarian tumor. L. B. AREY, Department of Anatomy, Northwestern University.

Many years elapsed, following its first presentation by Macnaughton-Jones (1898), Orthmann (1898) and Brenner ('07), before the so-called Brenner tumor of the ovary was properly recognized as an entity separate from granulosa tumors and the like. In these later years several sources of origin have been suggested; such include ovarian epithelium, epoöphoron, rete ovarii, mesonephric tissue, etc. No direct proofs have been advanced for such claims, but rather circumstantial relationships have been cited.

Two young specimens of Brenner tumor, in serial section, have been made available to me. In both, reconstructions show a direct stalk-like connection between the tumor-mass and the germinal epithelium of the ovary. The site of origin is a thickened, epithelial plaque. In the smaller tumor, four sprouts are growing into the ovarian stroma; only one connects with well-developed tumor cords. In the larger tumor there is one, relatively long, slender stalk that connects the surface epithelium with a complex, continuous system of epithelial cords that constitutes the tumor proper; another tiny epithelial process occurs on the inner surface of the plaque, but it is only a slight epithelial wedge.

There can be no doubt in these two specimens of the direct connection between tumor and surface covering of the ovary. Since most, if not all, of the previous reports on this tumor have been based on random, sample sections, it is easy to see how such connecting stalks could escape attention.

The venous return from the head and neck. O. V. BATSON, Department of Anatomy, Graduate School of Medicine, University of Pennsylvania.

X-ray examination and surgical exploration of a patient disclosed a complete occlusion of the superior vena cava and the vena azygos major. X-ray phlebograms show the venous return normal to the superior vena cava is now by way of the vertebral vein plexus. Eighteen months after the acute onset, the subcutaneous trunk veins have enlarged and taken over part of the return. It is to be assumed, on the basis of the anatomy of the venous system and of the studies noted above, that in those human cases where both internal jugular veins have been ligated simultaneously the vertebral vein plexus provides the immediately compensating channel.

With the presence of a plexus system of a caliber sufficient to function in these emergencies, it is evident that the vertebral channels function under normal physiologic conditions, such as during expiration. An experimental approach was tried in the cadaver by injecting one of the venules on the surface of the thyroid gland. The injected mass emerged into the superior vena cava and also into the vertebral vein plexus. Repeating this injection after increasing the intrathoracic pressure, the flow was principally into the vertebral vein plexus. Injections into brachial veins in the living human show that during expiration the flow tends to cross to the vertebral veins through named and unnamed channels similar to the transverse cervical and scapular veins. This throws new light on the function of these transverse veins. We must conclude that the vertebral vein plexus is a normal integral part of the mechanism for the return of blood to the heart.

Thalamic damage after asphyxia at birth. R. F. BECKER, W. F. WINDLE and Elsie F. HUNTER, Department of Anatomy and Institute of Neurology, Northwestern University.

Damage was encountered more often in the geniculate bodies and other thalamic nuclei than in any other parts of the brain in guinea pigs asphyxiated and resuscitated at birth. Survival time ranged from 2 hours to 3 months in 60 animals. In a third of these, damage was limited to the thalamus alone without involvement of the striatum or cortex. There was evidence either of hemorrhage about the anterior thalamic vessels, or of extensive nerve-cell degeneration beginning anteriorly and progressing posteriorly and ventrally to encroach upon or involve most of the geniculate bodies. While these cytopathologic changes were bilateral, the medial thalamus and most of the hypothalamus were spared.

A stereotyped pattern of sensory and motor sequelae characterized the recovery phase. The animals exhibited chorioathetosis, incoordinate righting and locomotion, hyperirritability, and hyperesthesia. They lacked startle reflexes to sound, and nuzzle reflexes necessary for feeding. When lying in the lateral position the undermost extremities were extended, the uppermost flexed. Motor recovery was gradual, and hyperirritability gave way to a permanent hypoesthesia with periods of marked somnolence. Impairment of vision and hearing correlated with geniculate damage. The general dimming of sensory modalities may account for the poor performance of these animals in simple learning tests as compared to the superior performance of unasphyxiated, littermate controls.

The lyssa of the dog. George A. BENNETT, The Daniel Baugh Institute of Anatomy, Jefferson Medical College.

The lyssa of the dog's tongue was studied experimentally and morphologically. By means of careful dissection the lyssa was exposed in six anesthetized dogs and in each instance a branch from the hypoglossal nerve was found running to it. Stimulation of this branch affected a visible elongation of the lyssa of about 1–2 cms. After the removal of the lyssa the result of immediate stimulation of the various branches of the hypoglossal nerve exhibited no appreciable variation in the contraction of the muscles stimulated.

The histological study of the lyssa revealed it to be composed of a definite connective-tissue capsule within which are nerve-fibers and striated muscle-fibers. The parallel arrangement of the muscle-fibers together with the capsule and nerves suggest that the lyssa may act as stretch afferents or a muscle spindle.

The cerebral cortex of the Galago. Gerhardt von BONIN, Department of Anatomy, University of Illinois.

The cortical areas of the brain of Galago demidovii were studied in a transverse and a sagittal series, stained alternately with cresyl violet and protargol. The brain is almost smooth. Its ventricle lacks a posterior horn. On the basis of thalamo-cortical afferents and corticocortical connections the primate cortex can be subdivided into regions. This scheme is employed in the following description of the cortex of Galago. In the occipital region, areas 17, 18 and 19 can be discerned. The Sylvian region shows areas 42 which is koniocortex on the supratemporal plane, 22 on the superior temporal convolution and 44 and 43 on the fronto-parietal operculum. In the central region, areas 6, 4 and 3 can be recognized. The parieto-temporal and the frontal region can not be subdivided into areas. The orbito-temporal region consists of the orbital area 47 and an undifferentiated temporal part. The temporal pole is covered by allocortex. Area 24 and a graniferous retrosplenial area fill the gyrus cinguli. (To appear in extenso in the Illinois Monographs in the Medical Sciences.)

Connective tissue cell counts as an index to tissue differentiation. Lester L. BOWLES, Department of Anatomy, University of Georgia School of Medicine. (Introduced by Joseph KRAFKA, JR.)

Cell counts were made in sections of the various connective tissues from twenty-two sites in the adult, fourteen in the newborn, eleven in 220 mm., seven in 18 mm., and four in each of 7.5 mm., 4.5 mm., and 2.5 mm. embryos. All the embryos and most of the older tissues were human. All were routine paraffin sections, cut at 10 micra and stained with hematoxylin and eosin. Counting was done in a calibrated square-ruled eyepiece disk at a magnification of 900 ×. The figures listed are the number of fixed cells per square millimeter of 10 micra section.

The highest cellularity in any area in the embryo is seen at the time of greatest activity in the laying down of the definitive structures to be derived from that area. From this time to the adult there is a constant and relatively uniform decrease in cellularity.

The possible error from mechanical factors is recognized. Yet, the counting technique used here is believed to be more readily adaptable and more accurate than other current methods of estimating degree of tissue differentiation.

It is suggested that the actual cellularity of connective tissue, as determined by cell count, may serve as a dependable aid in the determination of benignancy or malignancy on neoplasms arising in connective tissues. Such studies are under way.

The distribution of nerve cells and fibers to the choledochoduodenal junction of the cat. Edward A. BOYDEN and Charles VAN BUSKIRK, Department of Anatomy, University of Minnesota.

Degeneration studies of the nervous pathway to the choledochoduodenal junction, based upon serial sections stained with the Bodian technique, indicate that severance of both vagi and the peripheral branches of both celiac ganglia virtually eliminates fibers in the gastroduodenal nerve (the principal pathway to the junction) without appreciably decreasing the number of fibers that supply the duct after it enters the duodenum. As the choledochal ramus of the nerve approaches the junction it breaks up into small branches which either lose themselves in the pericholedochal plexus described by Schulze and Boyden or pierce the duodenal wall to join the myenteric plexus. In the submucosa, ganglia may be found subepithelially along the duct and in the muscle of the sphincter as well as in the submucosa. Terminally, in the papilla, ganglia occur subepithelially and in the muscle of the papilla. Finally, these subepithelial and sphincteric muscle nets which represent a continuation of the extramural plexus of the ducts, are in frequent communication with the myenteric and submucosal plexuses of the intestine. Surprisingly, in view of the supposedly more important function of the vagus, sympathetic components of the gastroduodenal nerve greatly outnumber vagal components. The former include some myelinated afferent fibers which may be traced to the mucosa of the intramural portion of the duct and to pacinian corpuscles found along its extramural course.

Seasonal incidence of anestrus in the postpartum rabbit.[1] James T. BRADBURY, Bureau of Dairy Industry, Agricultural Research Administration, U. S. Department of Agriculture.

The postpartum rabbit has been used for the assay of gonadotrophic substances. In the 12 months from June 1942 through May 1943 the incidence of anestrus was recorded for 156 rabbits having litters in this laboratory. The anestric condition was diagnosed at the time of laparotomy or autopsy within 2 to 4 weeks after parturition. Fifty per cent of the rabbits having litters in October and November became anestric. The lowest incidence of anestrus (6%) was noted in rabbits littering during February and March.

The average weight of the pituitary in the anestric rabbit is 32 mg. (range 24–39), in estric rabbits the average is 50 mg. (range 36–71). The anestric ovary averages 0.37 gm. (range 0.21–0.47), the estric ovary 1.02 gm. (range 0.47–1.80). The anestric uterus averages 5.4 gm. (range 1.5–8.0), the estric uterus 14.9 (range 9.5–20). These changes in organ size in the domestic rabbit are of the same magnitude as those found in the estric and anestric cottontail rabbit (Elder and Finerty, 1943).

The small size of the anestric uterus and the large size and tonic condition of the estric uterus makes it possible to judge the sexual condition of the rabbit quickly and quite accurately by abdominal palpation.

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[1] This research was supported by an appropriation from Bankhead-Jones funds (Bankhead-Jones Act of June 29, 1935).

Pigmentation of certain mesencephalic tegmental nuclei in the dog and cat. James O. BROWN, Department of Anatomy, University of Pennsylvania. (Introduced by Eliot R. CLARK.)

Sections of the brains of three dogs and three cats prepared for an earlier study of the pigmented substantia nigra and locus coeruleus (Brown, '43b) also showed pigment within neurons of the red nucleus, the oculomotor nucleus and the nucleus of the mesencephalic root of the trigeminal nerve. This pigment, assumed to be melanin, is brown in color in substantia nigra and locus coeruleus but it appears more yellowish in the other mesencephalic nuclei in unstained material. Impregnation with silver by the methods of Masson makes this argentophilic substance clearly visible.

From a comparative aspect, a report of the previously undescribed pigment in these mesencephalic tegmental nuclei of carnivores is significant.

Transplantation and subsequent development of various levels of the embryonic chick spinal cord in the presence of a growing limb. E. D. BUEKER, Department of Anatomy, Medical College of the State of South Carolina. (Introduced by A. M. LASSEK.)

Cervical, thoracic, and upper lumbar spinal cord segments of 20–30 somite chick embryos were removed in situ from the donor by use of the glass needle technique. These pieces, usually of five segments, were then transferred to a host of approximately the same age and implanted between the primordium of the innominate bone and the sacrum. The control for each of the above three series of experiments consisted of an embryo of the same age as the donors. In these a lesion was placed in the left half of the spinal cord immediately above and below that portion of the spinal cord to be transplanted. Shell fragments were placed in the lesions to prevent regeneration. These controls were also used to identify the segments of the transplantd cords. After a total of 9 days incubation, transplants were recovered, sectioned at 10 μ, and stained with Heidenhain's hematoxylin or by Bodian's method.

From 100 transplantation experiments, 18 were recovered with well developed limbs. In one of the best of the wing level series, segments 12–16, a total of 3200 motor neurons were found on the limb side of the transplant and 203 on the opposite side. Segments above the wing level and those segments between those supplying the wing and leg did not develop a motor column. However, near the issuance of the ventral roots a few scattered large multipolar neurons were found. These were in the position where the lateral motor column normally takes form and may have developed in response to the limb. As a result of the limb influence, the spinal ganglia of the various levels were found to have undergone a considerable hyperplasia, and the dorsal sensory columns were slightly larger.

Electro-architectonics — a correlative study of structure and function in the central nervous system.[1] Berry CAMPBELL, Department of Anatomy, University of Minnesota.

The peculiar and complex structure of the central nuclei has hitherto lacked functional interpretation because of absence of methods of investigation. It has long been suspected that the effect upon neighboring units of the potential fields generated by active neurons is the key to nuclear structure. So complex and interwoven are the potentials recorded with micro-electrodes with respect both to time and space that a simple method of applying this concept is necessary. The following procedure has been used with success.

Using micro-electrode manipulator, records are taken of evoked potentials at numerous stations. From the projected curves, the potentials are measured at chosen intervals (e. g., by msecs.). These figures are plotted on a series of maps traced from slides showing the needle tracks and the isopotential contours are drawn. The result is an animated view of the potential fields. These are found to rise and decline with time and to move in space in a way clearly correlated with the structural make-up of the centers. It is proposed to call such study of the functional arrangements of nuclei electro-architectonics.

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[1] Aided by a grant from the National Foundation for Infantile Paralysis.

A. An electro-architectonic study of the spinal cord following retrograde chromatolysis.[1]
B. The electro-architectonics of the inferior colliculus in response to auditory stimulation.[1]
Berry CAMPBELL, Department of Anatomy, University of Minnesota.

A. Systematic exploration of spinal cords of animals in which the tibial nerve had been previously sectioned was made with needle electrodes. Stimuli were evoked by stimulating the normal and the degenerated nerve. Antidromic conduction was prevented by ventral root section. The sequence and spacial distribution of the potential fields on the normal and the chromatolysed sides were compared. Conspicuous lowering of the potential gradient from the medial to the lateral side of the chromatolysed ventral horn was found at the time of and following the arrival of the presynaptic volleys. This was correlated with the absence of the proprioceptive reflex spike.

B. The inferior colliculus was investigated in a series of cats and rats by means of micro-electrodes. Clicks were used to evoke the potentials. The temporal course of stimulation of the secondary auditory pathways, of the dorsal nucleus of the lateral lemniscus and of the inferior colliculus itself was followed, and the characteristic series of potentials from each studied. The movement of the potential fields from the base to the dorsal surface of the nucleus of the inferior colliculus is shown on a series of contour maps of the colliculus. These are interpreted in support of the bipolar model of the nerve cell.

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[1] Aided by a grant from the National Foundation for Infantile Paralysis.

Sudden destruction of motor end plates by lactic acid. Eben J. CAREY and Leo MASSOPUST, Department of Anatomy, Marquette University.

Both sternomastoid muscles were exposed in living white rats. The right muscles were removed and used as controls. Into the exposed innervation of the left muscle 0.1 cc. of 0.05% lactic acid was injected in 10 rats; 0.1 cc. of 0.10% lactic acid into the muscle of

10 rats; and 0.1 cc. of 0.30% lactic acid in the muscle of 10 rats. Five rats were killed in each series within 30 seconds and the remaining 5 in each series at 25-minute intervals. Lactic acid in concentrations of 0.05 to 0.3% injected into the exposed zone of innervation of the sternomastoid muscle in the white rat produced sudden destruction of most of the motor end plates within 30 seconds to 25 minutes dependent upon the concentration of the acid. This liquefaction of the hypolemmal axons of the end plates was preceded by first a phase of retraction and then one of expansion and fragmentation. There was a progressive depletion of both the hypolemmal and epilemmal axons of their gold staining substances by lactic acid. While the hypolemmal axons were undergoing depletion by lactic acid, multiple gold staining fusiform bodies, 10 to 100 microns in length, appeared in many muscle fibers. The blood vessels were enlarged and congested with clumps of red blood cells.

Sudden destruction of motor end plates by lactic acid. (Demonstration). Eben J. CAREY and Leo MASSOPUST, Department of Anatomy, Marquette University.

Miscroscopic demonstration of the destruction of the motor end plates by lactic acid in gold impregnated preparations in comparison with the innervation in the normal controls of the sternomastoid muscle of the white rat.

Growth and behavior of epidermis as observed microscopically in the living, in chambers introduced in the rabbit's ear. Eliot R. CLARK and Eleanor Linton CLARK, Department of Anatomy, University of Pennsylvania.

Among numerous observations on the new growth and behavior of epidermis in thin (40–100 μ) spaces enclosed on two sides by rigid walls, the following are among the most interesting.

1. The extension, by cell migration, of a line of new epidermis is slightly more rapid than the extension of a growing line of fibroblasts and blood capillaries. Rapidly migrating epidermal cells closely resemble fibroblasts in shape.
2. Small groups of epidermal cells may become completely isolated, forming islands which eventually are completely surrounded by blood-vessels and connective tissue.
3. Fibrin that is near newly differentiating epidermis dissolves (fibrinolytic enzyme?).
4. Epidermal islands differentiate, forming fairly typical appearing epidermis, with growing basal layer oriented toward the blood capiliaries, and flattened, discarded layers at the center.
5. The blood-vessels do not penetrate newly formed epidermis. The nearest vessels are always very wide, with extremly active circulation.
6. Epidermal islands in the thin enclosed space of the chamber do not long survive. In less than a week the epidermal cells become vacuolated and die, the island is gradually invaded by blood capillaries, and the remains slowly removed, largely by the phagocytic activity of macrophages.
7. The connective tissue which forms next the epidermis is coarse and arranged in regular, parallel rows in contrast with the finer, irregularly arranged connective tissue fibers present elsewhere. This probably indicates a genetic difference between the dermal and the subdermal connective tissues.

The electroencephalograph in concussion and related states in the cat.[1] Sam L. CLARK and James W. WARD, Department of Anatomy, Vanderbilt University.

The electroencephalograph in normal and unaesthetized cats was established with implanted electrodes. Concussion was produced in some cats by a blow to the head with a falling weight; in others by the effects of dropping a weight on a plunger fitting loosely into a fluid-filled cylinder screwed into the cat's head and which communicated with the subarachnoid or epidural space. A third group of animals was immersed in one end of a water-filled U-tube and subjected to an impact produced by dropping a weight on a wooden float in the other end of the tube. These animals did not experience concussion but died within a minute or two from pulmonary hemorrhage.

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[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Vanderbilt University.

The E.E.G. in the first two groups showed a marked artifact at the time of the impact which was a slow wave of high amplitude lasting a little more or less than a second. This was followed by slow waves of lower amplitude than the original normal when the animal was deeply anaesthetized. The record approached normal near the time when the animals' reflexes returned as the concussion subsided. The record of animals subjected to water immersion impact showed a marked artifact at the time but the record cleared promptly and remained normal until it was altered as the animal became moribund from the pulmonary hemorrhage.

Effect of progesterone and desoxycorticosterone acetate on the x-zone of the mouse adrenal. H. J. CLAUSEN, Department of Anatomy, University of Colorado.

The purpose of this experiment was to determine whether progesterone or desoxycorticosterone would cause a degeneration of the x-zone of the mouse adrenal.

Daily intraperitoneal injections of 0.5 mg. of crystalline progesterone in oil were administered to one series of mice and daily intraperitoneal injections of 0.5 mg. of desoxycorticosterone acetate were given to another series of mice. Each group consisted of a series of young female and young castrate male mice. All animals were unilaterally adrenalectomized previous to the administration of the above steroids. After 40 daily injections, the animals were sacrificed and the remaining adrenal was removed for histological study.

The results show that progesterone as well as desoxycorticosterone acetate, when administered in the above dosage, causes partial degeneration of the x-zone of the adrenal. Complete vacuolization of the x-zone was not observed in any of the glands. The persisting cells of the x-zone appeared shrunken and irregular in outline and crowded together. Pyknotic nuclei were numerous and scattered vacuoles appeared between clusters of irregular cells. nuclei were numerous and scaattered vacuoles appeared between clustrs of irregular cells. Sinusoidal arrangement was replaced by condensation of cells. The adrenals of the females seemed to be more responsive to the steroids than those of the castrate males. No compensatory atrophy of the fascicular layer of the cortex was noted.

Neurons on the anterior roots of human spinal nerves. J. LeRoy CONEL, Department of Anatomy, Boston University.

Physiologic experiments have indicated that afferent fibers may be present in the anterior roots of spinal nerves. Cells in small numbers have been observed on these roots by a few investigators. In the present investigation human spinal cords of ages extending from birth to 3 years have been examined. Neurons have been observed on the anterior roots in all the cords. The cells are located near the ventral margin of the cord and in the ventral rootlets immediately outside the cord. These aberrant cells are relatively few in number, are most numerous in the younger ages, and are least numerous in the 3-year cord. They are especially numerous in cords of infants who had amyotonia congenita. All the neurons are in a state of degeneration as to chromophil substance, neurofibrils, dendrites, and axons. The signs of degeneration become more pronounced as aging advances. The neurons are probably not functioning.

Brain- and body-weight in man: Its antecedents in growth and evolution. Earl W. COUNT, Department of Anatomy, New York Medical College.

The human fetus, like those of other mammals (some primates, carnivores and ungulates) grows rapidly and fairly constantly in log brain weight with respect to log body weight. Some time after birth, the ratio is reestablished as constant but much lower; a transitional curve runs between the two phases. Phylogenetically, for log brain-weight/log body weight a second degree parabola, concave downward, can be fitted severally to the Primate (including man) and other mammalian stocks or orders. This denies the Dubois formula, also von Bonin's. The parabolae of the stocks converge back upon the Insectivora and Reptilia. All parabolas are mathematically consistent inter se. Man's brain development emerges as quite orthodox in the mammalian logic — not as aberrant. There is notable parallelism between the quantitative curves of ontogeny and phylogeny in the mammalian lines tested.

The effects of ether anesthesia upon the total erythrocyte and white cell counts of the adult female rat. Roger C. CRAFTS, Department of Anatomy, Boston University.

The use of anesthesia in blood work has long been discouraged. Contraction of the spleen and hemoconcentration have been reported in dogs after ether administration, while rabbits and cats showed no such effects. A previous study showed the normal total erythrocyte count for Long-Evans adult female rats to be 8.26 (± 0.68) million cells per cu. mm. In this study, twenty-one adult female rats were lightly anesthetized for less than 2 minutes on three separate occasions. The average erythrocyte count for these sixty-three samples of blood was 8.38 million cells, compared to a value of 8.39 million on sixty-three samples with no anesthesia whatever. Ether anesthesia for long periods of time likewise produced no variation from normal values. Blood was removed from rats after 2, 6, 10, 20, 30, and 60 minutes of anesthesia and showed average erythrocyte values of 8.37, 8.33, 8.20, 8.02, 7.96, and 7.84; all within normal range. Also, differences in depth of anesthesia produced no change from normal. Fourteen rats lightly anesthetized for 15 minutes showed an average total erythrocyte count of 7.84 million cells; deeply anesthetized rats for 15 minutes, 8.11 million cells. Total white cell counts showed no change in any part of the above work. It seems, therefore, that the number of formed elements in the blood of adult female rats of this strain is not altered by ether anesthesia.

A comparative study of the innervation of the pars distalis of the hypophysis cerebri. Glenn A. DRAGER, Department of Anatomy, Universities of Minnesota, West Virginia, and Texas. (Introduced by A. T. RASMUSSEN)

The hypophyses of man, ox, cat, dog, rabbit, rat, mouse, porpoise, chicken, and sparrow were prepared according to silver impregnation methods. In the anterior lobe of man, ox, cat, dog, mouse, rat, and rabbit, the majority of the nerve fibers are of autonomic origin. Such fibers are more numerous at the periphery of the gland and retain close association with blood vessels. In addition, an occasional aberrant fiber from the hypophyseal stalk passes to the pars distalis. In the porpoise and bird where the pars distalis is completely separated from the processus infundibuli, no nerve fibers were found in the anterior lobe. The only route by which hypothalamico-hypophyseal fibers could enter the pars distalis would be by way of the intervening pars tuberalis. Although the pars tuberalis receives both autonomic and hypothalamic fibers, no nerve fibers pass on into the pars distalis. No well defined arteries exist in the pars distalis, particularly in the bird. This may account for the absence of fibers here. No hypothalamico-hypophyseal nerve fibers were found in the pars distalis of the bird and porpoise, although the morphology of the gland in these forms facilitates the study of such nervous connections. Prominent sinusoidal vascular channels between the distalis and the tuberalis suggests a humoral rather than nervous control of the anterior lobe in these two forms.

Studies on the motor cells of the spinal cord. III. Position and extent of lesions in the nuclear pattern of convalescent and chronic poliomyelitis patients. H. Chandler ELLIOTT, Department of Anatomy, University of Toronto.

Seven human and one monkey cord were cut in serial section and studied in detail. In all cases dorsal and medial nuclei suffered first and most severely. Each lesion appeared to radiate from a single focus, of which there were a limited number in each subject. Evidence was found as to the functions of certain motor nuclei. These findings offer material to enable the clinician to visualize what is going on in the cord of a patient, and they narrow down the choice of paths by which the virus may reach the ventral grey column.

Numerical and regional distribution of taste buds on the tongue of the opossum, with particular reference to those in the fungiform papillae. Rush ELLIOTT, Department of Zoology, Ohio University, Athens, and E. B. Morris Biological Farm, The Wistar Institute.

A study of the numerical and regional distribution of taste buds on the dorsum of the tongue of the opossum, Didelphys virginiana, with particular reference to those in the fungiform papillae, has been made. The ages covered from 9 days postnatal through 135 days, with series taken at approximately 10-day intervals from the 25- through the 95-day series, and at 20-day intervals thereafter. Buds first appear in the 16-day series and there is a gradual increase in number through the 115-day series, with a decrease after that age.

The dorsum of the tongue from its cranial tip to the circumvallate papillae may be divided into fourths in relation to distribution of buds, with the greatest number in the middle two-fourths in all series studied. Few buds are present in the cranial fourth preceding the 75-day series, with a marked increase thereafter, and few are present in the caudal fourth before the 45-day series when the foliate papillae appear in this region.

Histological mappings prepared to show the position of the fungiform papillae in which buds are located reveal four distinct patterns of distribution: the first including 25- and 38-day series, the second the 45- and 56-day series, the third the 65- and 75-day series, and the fourth the remainder of the series studied.

The duration of estrus in rats with the bile ducts ligated. Frederick E. EMERY. Department of Physiology, University of Buffalo.

The duration of estrus after large doses of theelin and stilbestrol was determined in oophorectomized rats and compared to similar data taken from rats after the bile duct was ligated and severed. Four groups of rats were used. Each group was subdivided into a control series and a series with severed bile ducts. Theelin was given subcutaneously in one group and intraperitoneally in a second group. The controls receiving the theelin subcutaneously were in estrus for an average of 11.5 days as compared to 16.5 days in the series with bile ducts cut. In those injected intraperitoneally comparable figures were 5.0 and 4.6 days for the controls and experimental series respectively. Stilbestrol was given subcutaneously in the third group and intraperitoneally in the fourth group of rats. Here again those rats with the bile ducts cut were in estrus for a longer period of time. From these data it seems likely that either these estrogens are stored and later absorbed from the bile in the congested bile ducts or the damage to the liver prevents the usual destruction of these hormones. Since estrogens given intraperitoneally were destroyed in a few days in rats with severed bile ducts one is inclined to believe that this function of the liver is not greatly impaired. It, therefore, seems that the absorption of estrogen from the bile offers one explanation for the longer duration of estrous in rats with severed bile ducts.

Arterial lesions in rats with experimental hypertension. Victor M. EMMEL, Department of Anatomy, University of Rochester.

Hypertension was produced by the following methods: (a) collodion and gauze, (b) silk bag applied to one or both kidneys, or (c) silver clip partially occluding left renal artery. The latter technique gave lowest mortality and highest (60%) incidence of persistent hypertension. Cross and microscopic lesions in small arteries and arterioles, which developed in 65–75 percent of hypertensive animals, were most prominent in the mesenteric vessels.

The earliest lesions appearing in serial biopsy specimens show focal medial necrosis accompanied by marked adventitial edema and subendothelial accumulation of "hyalin" exudate. This is followed by polymorphonuclear infiltration of the entire wall and adventitial fibroblastic proliferation. Acute inflammation is gradually succeeded by marked proliferation in any or all layers of the vessel wall. The thickened wall sometimes becomes necrotic and distended with aneurism and thrombus formation. The lesions gradually disappear in animals whose blood pressure returns to normal after removal of the operated kidney, but progress if hypertension persists after nephrectomy.

Hypertension is not in itself sufficient to cause the lesions, since they are absent in some animals with chronic hypertension (200 days). Renal insufficiency may be a contributing factor, but lesions occur in animals with normal blood N.P.N. and normal phenol red elimination. Disappearance of lesions when a return to normal blood pressure follows nephrectomy suggests that a "toxic" factor of renal origin may be involved.

Striate body functions. Tests to determine uses of motor patterns (second report). Gertrude S. EVANS, William Waldo Blackman Laboratory of Anatomy, The New York Medical College. (Introduced by Thomas Horace EVANS.)

Further studies upon special individuals, medical students, defense-plant war-workers, policemen, business men. The test figures and diagrams bring out reactions of choice, or patterns which persist, applied to new or successive diagrams. Cortical interference noted. It seems the succession order of serial figures and diagrams affects use of motor pattern more strongly in certain persons.

A well disciplined policeman began the series of diagrams using one motor pattern, continuing it. Method of testing school-children (to appear in American Biology Teacher). Importance of studying test papers for line, direction, firmness, proportion, design. Lateral and controlateral influence of striate bodies, action of extrapyramidal tracts (abstract, Anat. Rec., v. 82, 1942, page 16; 1270 tests). Diagrams of circle, triangle, figure-of-eight, square and spiral. It is noticeable that rotation, once begun, attracts similar movements. Also, the figures: 1, 2, 3, 5, 7 have clockwise rotation. A shift to opposite rotation in known diagrams is easy; but unfamiliar, or facultative diagrams, are apt to fail to shift.

Association nerve tract between sixth encephalic (cranial) nerve and components of Meckel's ganglion through superior orbital fissure. B. *Incisive canal obliterated in human crania.* Thomas Horace EVANS, William Waldo Blackman Laboratory of Anatomy, The New York Medical College.

A. This important and interesting association nerve tract first seen by writer in 1918, studied. Luther Holden and John Langton make vague reference (Manual, 1879). A note to appear in American Journal of Ophthalmology.

B. Curiously, the incisive canal is often found closed, although in crania the nerve and vascular openings rarely obliterate. Edentulous conditions are usually present. The ganglion of Cloquet, and the nerve of Cotunnius, within the canal, have not been supposed to relate to the teeth of the premaxillary element. Nasal conditions considered; also general glandular stroma of anterior nasal fossa, including the "gland" of Stenson (Chauveau, Arloing and Fleming) with its ductus near the hiatus semilunaris. Relations of nasolacrimal duct in horse, ass, mule, dog, man. Region of "common colds." (1200 crania studied.)

Factors in interstitial-cell "repair" in the rat ovary. John W. EVERETT, Department of Anatomy, Duke University.

"Wheel" nuclei in interstitial cells of persistent-estrous rats suggest L H deficiency, yet the secretion of estrogen by these animals implies the action of L H. Repair of interstitial cells which accompanies luteinization induced in such rats by progesterone (Everett, 1941) is most pronounced in regions where extensive capillary beds are opened, for example, near corpora lutea. To explore this relationship between repair and circulation, an attempt was made to improve blood flow by interrupting the autonomic fibers accompanying the ovarian artery. Resecting ovarian vessels and adjacent tissue, thus leaving the ovary supplied with blood exclusively through uterine vessels, would presumably accomplish this. Fourteen persistent-estrous rats were so treated unilaterally, with the opposite ovary as control. Preserved at intervals of 1 to 35 days after operation, the experimental ovaries contained prominent capillary beds, increased tissue fluid and enlarged lymphatic channels. Interstitial cells became normal in appearance, beginning in about 3 days, while control ovaries remained unchanged.

Two normal female Vanderbilt rats were hypophysectomized and their ovarian vessels were unilaterally interrupted, as described above, 1 day after hypophysectomy. Seven days later, although the experimental ovaries showed increased tissue fluid and larger lymphatics, all ovaries exhibited "wheel" cells in equal degree. It may be supposed that the success of the operation in persistent-estrous rats indicates the presence of small amounts of L H, the effect of which is ordinarily obscured by ovarian ischemia.

The effectiveness of desoxycorticosterone acetate in adrenalectomized rats on a low sodium chloride diet. Wilburn J. EVERSOLE, Biology Department, The Rice Institute.

Male rats weighing approximately 55 gm. were adrenalectomized, fed various diets and injected subcutaneously with 0.2 mgm. desoxycorticosterone acetate (DCA) daily for 10 days. Fifteen rats were fed a purified diet consisting of dextrose, vitamin-free casein, salts free of sodium chloride, oils, and a rice bran concentrate. One of the fifteen animals died during treatment; the average survival was 12.5 days and the average weight gain per rat was 1.1 gm./day. Untreated, adrenalectomized rats, on a stock diet, survived for an average of 8 days and gained 0.7 gm./day. Each of eight intact control rats on the deficient diet averaged a weight gain of 2.9 gm./day as compared with an average gain of 5.2 gm./day for each of a group of ten rats on the stock diet. Animals on the deficient diet showed a loss of hair at 6–10 days. It appeared that these rats were suffering from both a vitamin and a sodium chloride deficiency.

The second diet was prepared as the first one except crystalline B-vitamins were substituted for rice bran concentrate. Of twelve adrenalectomized, DCA-injected rats but one died during treatment; the average weight gain per rat was 2.4 gm./day and the average survival was 14.7 days.

These experiments afford evidence that DCA is effective in the adrenalectomized rat when the salt intake is minimal.

Pattern of cyclic activity of women.[1] Edmond J. FARRIS, The Wistar Institute of Anatomy and Biology.

The walking activity of twelve healthy women, six married and six unmarried, was measured for 1 to 6 months. Three activity peaks were usually evident during the thirty-seven menstrual cycles that averaged 29 days in length, as follows:

A mid-interval activity peak on cycle day 15, with an average walking distance of 10.1 miles.

A menstrual activity peak on cycle day 4, with an average walking distance of 9.6 miles.

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[1] This investigation was aided by a grant from the Samuel S. Fels Fund.

A late cycle activity peak on day 24, with an average walking distance of 9.7 miles.

The average daily walking distance was 6.5 miles per cycle.

The walking activity of six male subjects for 1 to 6 months averaged 4.3 miles per day. No activity cycles were evident in any of the males.

Preliminary experiments based on running activity in female rats indicate the peaks of activity in the human female are hormonal in reaction, and are associated primarily with processes of reproduction.

An ovarian test for determining the probable ovulation time of women.[1] Edmond J. **FARRIS**, The Wistar Institute of Anatomy and Biology.

It is generally accepted that ovulation occurs usually during the mid-interval of the menstrual cycle, and that it is under hormonal control. Eight women were tested daily during cycle days 10–19 by injecting subcutaneously 2 cc. of morning urine of each in three immature rats. In addition, three of these subjects had daily samples run during their complete cycle. The rats were killed 2 hours after injection by exposure to illuminating gas in a closed chamber for 2 minutes. The ovaries showed red (positive) reaction on days 13, 14 and 15 of the cycle (range 12 to 18 days). An occasional red (positive) reaction occurred on 1 day during menstruation and also on about day 23 of the average 29-day cycle. On other days the ovaries were uniformly pale (negative reaction). If the color reaction of any of the three animals was markedly different from the others, two additional animals were injected.

A special color scale (Munsell system) permits accurate reading of color intensity, so that a definite idea of the amount of hormone causing the reaction is obtained. The positive reaction was most intense on day 15. This ovarian reaction is similar to the Salmon (U. J. Salmon, S. H. Geist, C. S. Poole, A. A. Salmon and I. L. Frank) reaction for pregnancy.

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[1] This investigation was aided by a grant from the Samuel S. Fels Fund.

The ratio between sensory and motor fibers in the chorda tympani and greater superficial petrosal nerves. James O. **FOLEY**, Department of Anatomy, University of Alabama.

It is well recognized that the chorda tympani and the greater superficial petrosal branches of the facial nerve are mixed nerves. The number of motor and sensory axons which each contains, however, has never been determined. This information has been secured for these nerves in the cat and dog by destroying the motor axons within or alongside the brain stem and later comparing the number of sensory axons in the degenerated nerves with the motor and sensory axons in the intact nerves.

The average ratio in the chorda tympani of the cat is 1.7 sensory axons for each motor axon whereas in the dog the sensory-motor ratio was 1.9 to 1.0. The average ratio in the greater superficial petrosal nerve of the cat is 1.5 motor axons for each sensory axon while in the dog the motor-sensory ratio was 2.6 to 1.0. Most of the nerve fibers of the chorda tympani, therefore, are sensory whereas the majority of those of the greater superficial petrosal nerve are motor.

The circulating leukocytes of suckling and non-suckling lactating mice. W. U. **GARDNER** and Jean H. **DOUGHERTY**, Department of Anatomy, Yale University School of Medicine.

The number of circulating leukocytes of rats was altered during changes in the functional activity of the mammary glands (Am. J. Anat., 38: 1–31). The studies reported here were undertaken to determine whether lactation affected the leukocytes of mice.

Young adult hybrid mice from four different crosses were used. All the mice were fed fox chow and water ad libitum. Blood was taken from the tail for differential and total W.B.C. and in some mice for R.B.C. The animals were divided into three groups as follows. (1) Twelve control non-lactating females or males from which counts were taken at 0, 24 and 28 hours.

(2) Eighteen lactating females (8–21 days post partum) from which the young were removed for 24 hours and then returned for 3 to 4 hours. Counts were taken at 0, 24 and 27–28 hours as above. (3) Ten lactating mice from which young were removed and not returned. Counts were taken as above.

The total white count of the controls did not change significantly, the means for the three stages being 13,790, 12,610 and 12,190. The lactating mice showed a marked leukopenia when nursing. Their mean counts were 7,480, 12,973 and 6,850 for the three periods. The lactating animals that did not have their young returned showed no recurrence of leukopenia. The leukopenia was due to a proportional decrease of both lymphocytes and polymorphonuclears. The red blood counts failed to show a significant alteration.

Osseous proliferation in estrogen-treated hypophysectomized mice. W. U. GARDNER and Doris H. CLOUET, Department of Anatomy, Yale University School of Medicine.

An extensive proliferation of intramedullary bone occurred in estrogen-treated intact mice (Proc. Soc. Exper. Biol. & Med., 37: 678; Anat. Rec., 76: 22: suppl.). Numerous investigators have demonstrated that skeletal growth was prevented by ablation of the hypophysis. The present experiments were undertaken to determine whether the pituitary was necessary for the osseous changes occurring in estrogen-treated mice.

Mice from 1½ to 3 months old were hypophysectomized and beginning 4 days later given 10 to 25 µg. of estradiol benzoate or dipropionate weekly for 4 to 6 weeks. Control hypophysectomized and control and estrogen-treated intact mice were observed simultaneously. All mice were fed Purina fox chow and water ad libitum. The formation of intramedullary bone occurred in the estrogen-treated hypophysectomized mice although less rapidly than in the intact animals. Histological sections of the femurs revealed increased osseous tissue in the bones of the estrogen-treated mice and chemical analyses revealed an increased ash and calcium content of the femurs. The total mean ash weight of the femurs of the hypophysectomized control mice was 23.32 mgm. (67.97%), hypophysectomized estrogen-treated mice 26.99 mgm. (69.92%), intact control mice 29.43 mgm. (67.97%) and intact estrogen-treated 37.38 mgm. (72.4%). The total amount and per cent of ash in the dry bones of the estrogen-treated hypophysectomized and intact mice was significantly larger than that of their respective controls. The osseous changes in the hypophysectomized mice show that estrogen may act upon the intramedullary bone in as specific a manner as upon the genital tissues.

Effects of thiouracil and para amino benzoic acid on the rat thyroid and resistance to reduced barometric pressures.[1] Albert S. GORDON, E. D. GOLDSMITH and Harry A. CHARIPPER, Department of Biology, Washington Square College, New York University.

The Mackenzies ('43) and Astwood ('43) have shown that thiouracil and para amino benzoic acid (PABA) are capable of inducing hyperplasia of the rat thyroid, a condition associated with a state of functional hypothyroidism; the former substance, however, has been found to be a more effective goitrogenic agent than the latter. It became of interest to subject this finding to a more detailed study. Adult male hooded rats were fed diets containing either 0.2% thiouracil (Lederle Laboratories) or 3% PABA (Merck). At the end of 19 days, the thyroid glands of six PABA-fed animals averaged 24.1 mg. whereas those from eight thiouracil-fed rats were 50.5 mg. After 40 days, the glands of seven PABA-treated rats averaged 37.3 mg.; those from six thiouracil-fed animals were 70.0 mg. The mean thyroid weight of eighteen normal untreated rats was 14.5 mg.

Histological studies revealed greater activity in the thyroids of the thiouracil-fed rats. Such glands possessed taller follicular epithelium and less colloid than those from PABA-fed rats. Although the pituitary glands from both types of experimental animals showed marked baso-

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[1] Aided by a grant from the Commonwealth Fund.

philia and the presence of numerous ''thyroidectomy'' cells, this condition was more accentuated in the thiouracil-fed rats.

Preliminary experiments have indicated that both thiouracil and PABA increase the resistance of rats to reduced barometric pressures (190 mm. Hg); in this respect, thiouracil has proved to be more effective than PABA.

An anomalous group of hamstring muscles. D. J. GRAY, Department of Anatomy, Stanford University.

In a white male cadaver, 84 years of age, the left semitendinosus, semimembranosus and long head of the biceps femoris muscles all arose from a common tendon. The tendon arose partly from the sacrotuberous ligament and was continuous with the fascia which passed cranially to split and ensheath the piriformis muscle. Inserting into the deep surface of the tendon were muscle slips arising from the ischium between the attachments of the obturator externus and the quadratus femoris, from the femur adjacent to the intertrochanteric crest and from the quadratus femoris muscle. The tendon was not attached to the ischial tuberosity, but was separated from it by a bursa. Distal to its origin the tendon became rounded, measuring 13 cm. in length and between 7 and 11 mm. in diameter.

The distal end of the tendon gave rise simultaneously to the muscle bellies of the semimembranosus, semitendinosus and long head of the biceps femoris, all of which passed to their insertions in the normal manner. The short head of the biceps femoris was not unusual either in its relation to the long head or in its insertion.

The tibial portion of the sciatic nerve supplied three branches which became lost in the substance of the common tendon. The semimembranosus received three branches; the semitendinosus and the long head of the biceps were each supplied with one branch.

Concussion of the spinal cord. R. A. GROAT, W. A. RAMBACH and W. F. WINDLE, Institute of Neurology, Northwestern University.

The back of the vertically hanging lightly anesthetized or spinal animal was struck with a wooden soft-ball bat. Concussion was deemed to have occurred when there was a rise in threshold of appropriate responses elicited by stimulation of the femoral nerve or the pyramids. Concussion can occur without any gross damage. The length of cord involved in concussion depended upon the force of the blow and could be observed in both the functional and histologic studies.

In animals killed 6 days after concussion chromatolytic changes were found among the large cells of the posterior horn and occasionally in the anterior horn. Many of the medium and small cells of the gray matter were affected. Correlated functional and histologic observations, and experience gained in brain concussion studies indicated that the interneurons of the spinal gray were principally involved in concussion. The functional studies showed that a block of long ascending and descending tracts is part of the picture of spinal concussion, though this is not true of brain concusion. [*The work decribed in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Northwestern University.*]

Atresia of anus with rectourethral communication. Béla HALPERT and Daniel L. TAPPEN, Laboratories of the University Hospitals, University of Oklahoma.

The absence of an anal opening and canal and a communication between rectum and urethra were observed in a white male child born near term who died when 10 days old. Absence of the anal opening was noted at birth and the third day after birth, passage of meconium following urination was observed. Four days later a ureteral catheter was passed through the urethra into the rectum and the existing communication was demonstrated roentgenologically.

The child died 3 days later. At necropsy it was 48 cm. tall and weighed 2,125 gms. The proximal jejunum was collapsed, the distal portion and the ileum were distended. Forty centimeters oral to the ileocecal region on the free margin of the gut there was a finger-like projection 3 × 2 cm. The colon was large and distended, particularly the left half and reached maximum dimensions in the sigmoid colon and rectum. The rectum ended blindly 2 cm. proximal to the skin surface. The urinary bladder contained clear urine. The trigone was intact. In the urethra below the colliculus seminalis there was an opening 0.2 cm. in diameter through which passage of fecal material was obtained by pressure on the rectum.

The effect of peripheral factors on mitotic activity and cellular differentiation of motor neurons in the chick embryo. Viktor HAMBURGER and Eugene L. KEEFE, Washington University.

Wing bud extirpation at 3 days of incubation results in a hypoplasia of the brachial lateral motor column. The question whether peripheral factors operate by blocking mitotic activity or histological differentiation was tested by counting the mitotic figures on both sides of the brachial level during the peak of mitotic activity. No convincing evidence was obtained for an effect on mitotic proliferation although the figures for the operated side are 2% lower than those of the control side.

If the effect is primarily or exclusively on cellular differentiation, then the sum total of motor and non-motor neurons should be identical for the hypoplastic and the normal side. This expectation is fulfilled. Lateral motor neurons and, separately, all other cells were counted in the ventral halves of the hypoplastic segments of two wing bud and two leg bud extirpation cases. The motor neuron hypoplasia amounted to 47% to 92%. In all cases the deficiency in motor neurons was compensated almost exactly by an excess in non-motor cells. The total difference between left and right side in four cases amounts to 0.15%. A case of motor hyperplasia, obtained by wing bud implantation showed likewise identical total numbers on both sides (1.1% difference). The peripheral factors operate apparently by controlling the number of cells of a pool of indifferent cells which are to differentiate into motor neurons.

Titers of urinary gonadotropins in old eunuchs who had been castrated prepubertally, and evidence of supranormal secretory activity of an endocrine gland for 40 to 50 years. James B. HAMILTON, Hubert R. CATCHPOLE, and Charles C. HAWKE, Departments of Anatomy, Yale University and University of Missouri, and from the Adolescence Study Unit and Laboratory of Physiology, Yale University.

Four men, who had been castrated between 8 and 12 years of age and at the time of the present study had been eunuchs for 44 to 51 years, excreted large quantities of urinary gonadotropins, 160, 160, 40 and between 40 and 80 M.U. daily. These values far exceeded the average daily excretion of 10–25 M.U. by ten normal men 55 to 76 years old.

The presence of large amounts of urinary gonadotropins in prepubertally-castrated eunuchs seems to date from the normal chronological age of puberty, if not slightly earlier, as judged by collateral studies of two boys castrated before the advent of manifest puberty.

If the elevated titers of gonadotropins in castrate individuals reflects an augmented secretion of gonadotropins by the pituitary gland, as is generally believed, a continued production of greatly supranormal quantities of gonadotropins during most of the lifespan of these eunuchs provides a striking illustration of prolonged hyperfunction of an endocrine gland.

Although primary gonadal insufficiency comparable to that in women past the menopause does occur in old men, as after bilateral orchidectomy or destruction of the testes, climacteric-like conditions would not appear to be either a usual or a normal sequela of ageing in men.

On the volumes of basal ganglia and neo-cortex in primates. Pinckney J. HARMAN, Georgetown University Brain Research Institute.

The volumes of the caudate nucleus, putamen, globus pallidus, neocortex and the total brain have been estimated in a series of primates extending from lemuroids to man. The volumes were obtained by applying a method of serial planimetry and include observations on galago, potto, Cebus, Ateles, Cerocebus, Macaca and Pan, as well as those on the human brain.

The impression created by some writers that the neo-cortex overshadows the basal nuclei in primate evolution receives no support from the purely quantitative results obtained in this study. All nuclear masses are roughly proportionate to total brain size so far as volumes are concerned. Moreover, the relationship between cortical volume and brain volume is such that it is possible to predict one value from the other with a fair degree of accuracy.

The only striking phylogenetic change observed occurs with respect to the caudate-putamen complex. The putamen is smaller than the caudate nucleus in the two lemuroids, but larger in the remainder of the series.

Whatever superiority the neo-cortex possesses in primates over the basal ganglia either is based on finer structure and function or has been derived at an earlier stage of development than can be discerned by a study of the brains in this series.

The afferent pupillo-dilator pathway in the cat. A. J. HARRIS, R. HODES, and H. W. MAGOUN, Department of Anatomy and the Institute of Neurology, Northwestern University.

The pupillo-dilator response to nociceptive stimulation has recently been shown to result in the cat entirely by inhibition of oculomotor constrictor tonus. Since this inhibition occurs at the rostral midbrain, the response has here been employed in following the afferent pathway concerned forward through the spinal cord and lower brain stem.

Observing the pupillo-dilatation to sciatic and splanchnic nerve stimulation after neural lesions in lightly anesthetized cats, it was found that the responses evoked from cutaneous and visceral afferents could not be dissociated, but rather appeared to be mediated by a common ascending pathway, whose distribution was distinct from that of the lateral spinothalamic tract at levels above the caudal medulla oblongata.

This afferent dilator pathway ascends in the lateral funiculus of the spinal cord and the reticular formation of the lower brain stem, gradually assuming a dorsal and paramedian position, to pass forward through the midbrain close to the midline, either in the ventral part of the central grey of the aqueduct or in the immediately adjacent mesencephalic tegmentum.

The pathway thus seems to be delivered directly to the oculomotor region; and, except for destruction of the pretectal area, which alters oculomotor tonus, extirpation of neural parts above the midbrain did not affect the response.

Seasonal variations in the structure of the anterior hypophysis of the garter snake. J. Francis HARTMANN, Department of Zoology, Cornell University. (Introduced by J. M. WOLFE.)

Differential counts were made of the cells in the anterior hypophyses of male garter snakes taken during each month of the year to determine the normal seasonal variations exhibited by these cells. With the technique of Dawson and Friedgood, it is possible to demonstrate four tinctorial cell types, referred to as carmine cells, orange cells, basophiles and chromophobes. The carmine and orange cells are considered to be acidophiles. The carmine cells in any given month number approximately 33% of all cells counted, except in the summer, when they reach a value of 50% (July) and 46% (August). The orange cells make up between 25% and 30% of all cells counted, except through the spring, when they rise sharply to reach a maximum of 43% in June. The basophiles are fewest in summer (4%) and in winter (6%), but reach a peak of 13% in spring and a second peak of 11% in the fall. The chromophobes decline steadily from a winter value of 33% to a midsummer value of 9%, and rise sharply in the fall to regain the winter level. The increased numbers of basophiles in the pars distalis in April and September may possibly be correlated with increased size of the testes and increased spermatogenetic activity at these periods.

Some interesting features in a double male human monster (thoracoomphalapagus). Edward A. HOLYOKE, Department of Anatomy, University of Nebraska.

This specimen viable at term died immediately after delivery. The components are equal in size and cephalad to the point of fusion are normal. They are joined ventrally from the fifth sternochondral junction through the composite deformed pelvis. There are two ''abdominal walls'', one ventrolateral in relation to the viscera of each component. The musculature originates from the respective fetuses and inserts into a common linea alba. A single umbilical cord containing a vein and two arteries perforates one ''wall''. There are three legs, one normal and one deformed. One of the two descended testes belongs to each component. The anus is imperforate.

There are four lungs, with separate pleural cavities, and two hearts with common pericardium. Hearts and great vessels are normal. The common diaphragm is broadly applied to the large composite liver. The gastrointestinal tracts are complete to a few inches from the single caecum where the ilia join. The peritoneal relations of the liver to each gastrointestinal tract resemble the normal. There are two portal veins, hepatic arteries, bile ducts and gall bladders. The hepatic diverticula evidently grew into a common septum transversum and secondarily fused. The falciform ligament transmits the umbilical vein which bifurcates, a ductus venosus leading to each vena cava.

Apparently this specimen represents early fission of the embryonic axis. Neither component shows any transposition of viscera (situs inversus).

The effect of estrogens on the mitotic activity of the rat hypophysis. Thomas E. HUNT, Department of Anatomy, University of Alabama.

An increased mitotic activity occurred in the hypophysis of ovariectomized rats 50 to 70 hours after single injections of alpha estradiol or theelin. Equal amounts by weight of the two estrogens gave comparable results. In animals 80 to 150 days of age there was only a slightly increased activity after injection of 15 to 20 gammas (average of nine animals, 3.8 mitoses per sq. mm. of section). In order to obtain mitotic activity comparable to that found in the late estrous stage of the normal rat of this age it was necessary to inject 150 to 200 gammas (average of eleven animals, 26.7 mitoses per sq. mm.). In older rats from 300 to 600 days of age injections of 150 to 400 gammas did not cause a significant rise in the mitotic activity (average of ten animals, 1.14 mitoses per sq. mm.). The fact that injections of estrogens result in increased mitotic activity of the genital epithelium 24 to 36 hours earlier than in the hypophysis, suggets that the effect on the latter may be indirect. The unresponsiveness of the cells in the older hypophyses confirms the conclusion made previously that they become increasingly refractory to mitotic stimulating factors as the animals become older.

On a normal human ovum of approximately 7½ to 8 days of age. Arthur T. HERTIG and John ROCK, Free Hospital for Women, Brookline, Mass., Departments of Pathology, Obstetrics and Gynecology, Harvard Medical School and Department of Embryology, Carnegie Institution of Washington.

The ovum (Carnegie, 8155) was discovered 24 hours after fixation in a uterus removed surgically on the twenty-second day of the cycle. Its implantation site was anterior, at the fundus and nearer the right side (corpus luteum left), appearing as a raised, irregularly oval ring 0.21 × 0.27 mm. in diameter and containing a 0.038 mm. embryonic mass.

Microscopically the endometrium is in the twenty-second day with marked stromal edema and secretory activity. The chorion measures 0.150 × 0.210 × 0.306 mm.; the chorionic cavity

0.066 × 0.082 × 0.168 mm.; the embryo 0.030 × 0.050 × 0.090 mm. and the amniotic cavity 0.020 × 0.032 × 0.048 mm. Predecidual reaction is absent about the ovum as elsewhere. Leucocytes are absent. Vascular response about the ovum is just beginning.

The trophoblast varies from the thin, indifferent blastocyst wall (abembryonic pole) to a thick shell of admixed primitive syncytio- and cytotrophoblast (embryonic pole). Active invasion of maternal stroma by irregular tongues of primitive syncytiotrophoblast is taking place. Vacuolization of the latter is just beginning.

The chorionic cavity is crowded by an eccentrically-placed, primitive bilaminar germ disk. The amniotic cavity is prominent due to concavity of the primitive ectoderm. Amniogenesis has not begun. A few primitive mesoblastic cells are delaminating in situ from adjacent cytotrophoblast. The exocoelomic (Heuser's) membrane has not appeared.

On a normal human ovum of approximately 8 days of age. Arthur T. HERTIG and John ROCK, Free Hospital for Women, Brookline, Mass., Departments of Pathology, Obstetrics and Gynecology, Harvard Medical School, and Department of Embryology, Carnegie Institution of Washington.

The ovum (Carnegie no. 8171) was discovered, after fixation, in a uterus removed surgically on the twenty-third day of the cycle. Its implantation site was posterior, near the fundus and also relatively near its ovary of origin, appearing as an ulcerated, slightly elevated area 0.57 × 0.42 mm. Transillumination revealed a darkened, honeycombed, oval structure beneath the elevation 0.30 × 0.46 mm. The endometrium is in its twenty-third day with active secretion and marked stromal edema.

The chorion measures 0.256 × 0.404 × 0.422 mm.; chorionic cavity 0.080 × 0.164 × 0.138 mm.; embryo 0.046 × 0.088 × 0.114 mm. and amniotic cavity 0.024 × 0.040 × 0.036 mm. Early decidual reaction occurs about the ovum accompanied by local leucocytic infiltration. Vascular response to the ovum has begun and several dilated, empty, capillary sinusoids are in continuity with syncytiotrophoblastic lacunae. The latter are in all stages of formation from tiny vacuoles to large multilocular spaces which are coalescing with their neighbors. Maternal blood occupies a few of them but most are empty save for a few leucocytes. The chorionic cavity barely accommodates an eccentrically situated bilaminar germ disk. The prominent amniotic cavity is formed by the concave primitive ectoderm below and the early amniogenic tissue above, the latter arising from adjacent cytotrophoblast. Heuser's (exocoelomic) membrane is just beginning to form, apparently delaminating in situ from adjacent cytotrophoblast.

Time relations and temporal facilitation of a trigeminal reflex pathway. William O. HILLE, Department of Anatomy, University of Minnesota. (Introduced by Berry CAMPBELL.)

The reflex pathways involving the lingual and the inferior alveolar nerves on the sensory side and the mylohyoid nerve on the motor side were investigated in cats. A cathode ray oscillograph and a sweep synchronized stimulator were used. The latency of weak shocks was 5 msec. This could be shortened to 3.5 msec. by increasing the stimulus strength. Conditioning with two moderate shocks on the inferior alveolar nerve showed facilitation beginning at 2.6 msec. Conditioning the inferior alveolar nerve by lingual nerve showed facilitation at 2.2 msec. These figures of temporal facilitation indicate a nuclear delay in the sense that the term was used by Lloyd.

Nerve cells in tissue culture. Mary Jane HOGUE, Department of Anatomy, University of Pennsylvania.

The brain of a new born pup was removed aseptically. Small pieces from the vermis, cerebellar hemispheres and the region of the pes pedunculi were cultured in roller tubes. At first there was a migration of small neuroglia cells. These did not live long. Later cells with long processes migrated out from the explants of the cerebellar hemispheres and of the pes pedunculi, and moved about in the surrounding fields. There were both pigmented and non-pigmented cells from the region of the pes pedunculi. Some of the cells had long slender processes; a few were multipolar and a few were pyramidal in shape. These were kept alive and studied for over 45 days. Serial photographs were taken of these nerve cells illustrating their power of locomotion.

Cell number — volume relationship in the medullary plate of Amblystoma punctatum. Cranford **HUTCHINSON**, The Daniel Baugh Institute of Anatomy, Jefferson Medical College and The Morris Biological Farm of The Wistar Institute.

In order to determine whether changes in volume or in cell numbers were correlated with the "spreading" or "stretching" movements seen in the developing medullary plate, three series of Amblystoma embryos fixed during neurula stages were measured for volume and total cell number using the method devised by Dornfeld, Slater and Scheffé, '42. The embryos came from three different clutches of eggs, were raised at a constant temperature, fixed at hourly intervals, and prepared for histological study in a manner as nearly uniform as possible. The number of cells per unit mass of medullary plate tissue was then calculated for embryos from Harrison's stage 13 through stage 16.

Considerable variation among the series was found but all showed a brief period in early stage 14 when the number of cells per unit volume falls perceptibly. This is when the spreading movements in the medullary plate are most pronounced as may be seen in vitally stained embryos; but there appears to be no clear relationship between the movements and the relative decrease in cell numbers, for the movements are most pronounced in the posterior plate region, while the proportional decrease in cell number is most marked in the anterior plate region.

Occurrence of cystic ovaries in thyroidectomized rats treated with diethylstilbestrol. Ralph G. **JANES**, Department of Anatomy, Wayne University.

Rats in our colony, Long-Evans strain, which have been inbred for many years, rarely exhibit cystic ovaries following estrogen therapy in any amount or for any duration. Twenty-three rats were thyroidectomized for a period of 8 months. The ovaries were examined in eight of these animals at the end of this period. One rat only had a cystic ovary. Five of these rats were given subcutaneous pellets of diethylstilbestrol at the time of thyroidectomy and again 6 months later. At autopsy only one rat of this series showed cystic ovaries, the ovaries of the other animals being somewhat atrophic. However, seven of the ten remaining rats, which were given daily subcutaneous injections of 100 micrograms of diethylstilbestrol for 20 days before killing had cystic ovaries. No cysts were seen in fourteen rats which had been thyroidectomized for 1 month and, in some instances, treated with diethylstilbestrol during the last 20 days.

The cysts were transparent structures easily seen with the naked eye. Microscopically they appeared as sacs filled with follicular coagulum and surrounded by connective tissue. Granulosa cells usually were absent in large cysts while the theca in some instances had undergone luteinization in certain areas.

It has been observed that in our rats a hypothyroid condition of several months duration, together with elevated estrogen levels for 20 days, will increase the incidence of cystic ovaries. With prolonged periods of estrogen stimulation, no increased incidence has been noted.

Effects of diets containing desiccated human and hog stomach preparations on embryonic rat erythropoiesis.[1] Oliver P. **JONES**, Department of Anatomy, University of Buffalo.

Twenty autopsied human stomachs were divided into pyloric, fundic and cardiac portions and prepared according to the method described by Fox and Castle (1942). Four of these were ground with an equal wet weight of beef muscle. Preparations of hogs pylori and cardias were furnished by Parke, Davis & Co. Some hog pylorus powder was boiled in water for 5 minutes. Diets containing 2.5% of these substances were fed to pregnant rats and studies of embryonic blood were conducted according to a previously described technic (Jones, 1942). Control series consisted of seventy-one embryos from thirty-five rats while the test series had 114 embryos from fifty-six rats. In general, results from these few experiments were in accord with observations made by Fox and Castle. Human and hog pylori were both significantly active but human pylori plus beef were inactive. Human fundi alone were inactive when ground beef was added. Hog cardias were active but human cardias with or without beef were inactive. Hogs' pylorus powder was inactivated by boiling in water. Extension of fundic glands into the pyloric region may account for the activity of human pylorus alone. Addition of beef muscle presumably diluted these preparations. Inactivity of human fundic preparations may be due to insufficient extrinsic factor in the musculature, which was apparently overcome by the addition of beef muscle.

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[1] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

The functional significance of the declination of the axis of the human talocalcaneonavicular joint. Russell L. JONES, Department of Anatomy, Indiana University.

The axis of the talocalcaneonavicular joint has long been illustrated in textbooks of Anatomy. Manter ('41) measured the declination of this axis finding it 42 degrees from the long axis of the foot in the vertical plane and 16 degrees in the horizontal plane. Little has been written of the functional significance of the peculiar declination of this axis. Appropriate measurements on fifty subjects show that, on account of the declination of this axis, rotational torque in the tibia affects greatly what Elftman ('34), Morton ('35), and Jones ('41) have shown to be a significant physiological attribute of the human foot — namely, the ratio of weight distribution among the five metatarsal heads comprising the ball of the foot. Lateral rotational torque in the tibia increases the proportion of weight borne by the lateral four metatarsal heads, while medial torque increases the proportion of weight borne by the first metatarsal. The effect on weight distribution is roughly proportional to the torque on the tibia.

Since the rotator muscles of the lower extremity strike a balance at a given position, varying in different subjects, the effect of rotational tibial torque on weight distribution provides a rationale for the therapeutic effects which can sometimes be achieved by alteration of the walking gait with respect to out-toeing, in-toeing, or walking with toes straight ahead.

Polycystic kidney disease in inbred mice. Arthur KIRSCHBAUM, Department of Anatomy, University of Minnesota.

Polycystic kidney disease has appeared in a majority of Strong strain A mice of the Minnesota stock living beyond 1 year of age. Grossly these kidneys have been either large and cystic, or deeply pitted with only small cysts, in the latter case being of normal size or even smaller. Occasional polycystic kidneys have been observed in old mice of other strains. Seventy-seven F_1 hybrid mice between strains A and F did not exhibit the condition. When F_1 hybrids were backcrossed to strain A, the disease appeared in seventeen out of forty-three animals autopsied (fourteen of the twenty-six negative animals died of leukemia). Cystic kidneys did not appear in any of forty-one backcross mice to the F strain. It appears that the tendency to develop polycystic kidney disease is in this instance inherited as a Mendelian recessive character. A series of kidneys of strain A embryos and strain A animals of different ages is being studied to determine the embryogenesis of the aberration.

Glomerulonephritis in inbred mice. Arthur KIRSCHBAUM, Department of Anatomy, University of Minnesota.

Eight grossly edematous mice of the Strong NH strain were studied. Edema fluid was present in the subcutaneous tissue, abdominal and pleural cavities. The kidneys were pale, but of normal size and weight. On histologic section they revealed glomerular alteration identical with that of human glomerulonephritis — thickening of the capillary basement membrane, closure of glomerular capillaries due to formation of intercapillary fibers, hyalinized glomeruli, capillary thrombosis, and epithelial crescents. In some cases not all the glomeruli were involved, whereas in others the process was general enough so that the term ''diffuse glomerulo-nephritis'' was applicable. Albuminuria was evidenced by casts in the tubules. Many tubules were dilated, but only a small degree of tubular degenerative change was present.

At least one-fourth of untreated strain NH mice of the Minnesota stock have developed the condition. Mice were obtained originally from Dr. L. C. Strong of Yale University School of Medicine; these mice had been inbred for eight generations. The age of clinical appearance of the disease has ranged from 5 to 18 months. Glomerular pathology was noted in one old male which gave no clinical evidence of kidney disease.

Since glomerulonephritis has not been previously observed in animals, the existence of this condition within an inbred strain of mice is considered to be of importance. Further studies are being pursued.

Senile changes in the brain of Wistar Institute rats. Hartwig KUHLENBECK, Department of Anatomy, Woman's Medical College of Pennsylvania.[1]

A study of the brain in the series of Wistar Institute rats ranging from birth to old age reveals senile changes of vascular apparatus, nerve cells and glia. The present communication is restricted to the findings concerning blood vessels and cortical nerve cells.

1. Vascular alterations affect mainly arteries and arterioles and consist of circumscribed as well as diffuse thickenings of the intima and degenerations of the media. The amount and thickness of adventitial connective tissue accompanying the blood vessels within the central nervous system has a tendency to increase with age. The argyrophil fibers in this tissue become more numerous and coarser.

2. Senile cortical cell loss.

During the last third of the rat's life span nerve cells in the entire cerebral cortex show in increasing number the typical picture of chronic ganglion cell disease with pyknosis and shrinkage. This change is especially pronounced in the third layer of the neocortex but is also noticeable in other layers as well as in archicortex, palaeocortex and basal cortex.

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[1] The study of Wistar Institute rats is part of a program on ageing organized by Dr. E. J. Farris of The Wistar Institute, with the support of the Samuel S. Fels Fund.

The sexual cycle and reproduction in the golden hamster (Cricetus auratus). Herbert S. KUPPERMAN, Robert B. GREENBLATT and L. Q. HAIR, Department of Experimental Medicine, University of Georgia. (Introduced by G. Lombard KELLY.)

The reproductive cycle of the golden hamster was studied for a period of 15 months. The cyclic changes in the vagina, as observed by vaginal lavage, are completed every 4 days without change or modification throughout the year. The cyclic manifestations in reference to ovulation occur as follows: A smear of cornified cells is observed prior to ovulation and may be considered as a proestrous smear. On the day following the appearance of cornified cells and associated with post-ovulatory changes in the ovaries (newly-formed corpora lutea), the vaginal smear is characteristic and may be recognized grossly as a dense, opaque, white, sticky discharge which microscopically is made up mainly of large, nucleated, oval cells and some cornified cells. The vaginal smears on the remaining 2 days of the 4-day cycle exhibit vast numbers of leucocytes. Vaginal introitus occurs between the tenth and fifteenth day of life but corpora lutea formation and cyclic changes in the vagina are not initiated until the thirty-third to the thirty-eighth day of life. The sexual cycle of the normal female hamster once initiated will continue regularly and uninterrupted until senility, which is associated with ovarian failure and may occur as early as 1 year but is usually observed in our colony in animals 400–500 days of age. Mating occurs, without exception, in the transitional period between the cornified cell stage and the thick, dense smear of nucleated and cornified cells. In seventy-one timed pregnancies, the interval between the observation of sperm and parturition was 14 days in 4%, 15 days in 85% and 16 days in 11% of the animals. In seventy-nine litters, there was an average of 9.7 young per litter with a sex ratio of 369 males to 401 females. The animals breed the entire year and show no evidence of a breeding pause or decrease in reproductive activity during the winter months.

Spermatogenesis commences about the thirty-fifth day of life and continues, regardless of season, until senility. This, as in the female hamsters, may be observed in animals 400–500 days of age.

Hormone control of a dimorphic pigmentation area in the golden hamster (Cricetus auratus). Herbert S. KUPPERMANN, Department of Experimental Medicine, University of Georgia. (Introduced by G. Lombard KELLY.)

On the dorosolateral subcostal region of the skin of the male golden hamster there is normally a dark, accentuated pigmentated area of about 5–7 mm. in diameter. The hair in this particular region in the male is coarser than that seen in the rest of the pellage. The female hamster may either show no pigmentation in this area at all or present only a minute area of slight pigmentation with no difference in hair texture. The effect of hormones upon this pigmentation spot was studied in the normal and castrated female and male hamster by implanting pellets of testosterone propionate, estradiol dipropionate, progesterone and desoxycorticosterone acetate (DCA) subcutaneously in the mid-scapular region. In the normal male, testosterone accentuates the degree of pigmentation while estradiol and castration caused its complete regression. DCA and progesterone brought about some decrease in intensity of the coloration. This action, however, is probably not a direct one but may be attributed to the indirect action of these compounds upon the testis itself by partially suppressing the hypophyseal gonadotropic complex. In the castrate male and normal and castrate female hamster, testosterone maintained the pigmented area or in the absence of pigmentation it induced its formation. DCA, progesterone and estrogen were without effect upon pigmentation in these animals. The pigmented area was absent in the new born hamster and first made its appearance in males at about the thirtieth to thirty-fifth day of life, when a marked increase in size of the male accessories also occurred. There was a decrease in extent and intensity of the pigmentation in senile male hamsters exhibiting signs of testicular failure.

A study of the length of first and twelfth ribs in 559 American Whites and Negroes. Raymond R. LANIER, Jr., Department of Anatomy, Washington University.

The first ribs of Whites are longer than those of Negroes. The first ribs of White males, Negro males and White female are longer than those of Negro females; there is no difference in length of first ribs in the first three groups. There is no difference in variability in length of first ribs between the races; first ribs of White females and Negro males vary more in length than those of White males and Negro females.

There is no racial difference in length of twelfth ribs. Twelfth ribs of males are longer than those of females. Twelfth ribs are longer than first ribs in White males, Negro males, and Negro females. Twelfth and first ribs are of equal length only in White females. Twelfth ribs vary in length more than first ribs, and in Whites more than in Negroes.

The length of the first ribs cannot be predicted from the length of the twelfth ribs, and vice versa.

Level of termination of the dural sac.[1] Virginia Singleton LANIER, Department of Anatomy; Howard E. McKNIGHT, Department of Obstetrics and Gynecology; and Mildred TROTTER, Department of Anatomy, Washington University.

The dorsal wall of the sacral canal was removed by appropriate saw cuts in fifty-six cadavers thus exposing the caudal extremity of the dural sac. Before removal of the wall the level of the apex of the sacral hiatus was marked by the insertion of a straight pin into the ventral wall of the canal; following removal of the dorsal wall the level of termination of the dural sac was marked in a similar manner.

The point of termination of the dural sac occurred between the levels of the middle of the first and the middle of the third sacral vertebral bodies. The mean point was the middle third of the body of the second sacral vertebra. The termination of the dura was at a level cephalad to the mean in 37.5% and caudad in 46.4%.

The distance between the lower extent of the dura and the apex of the sacral hiatus demarcated by the pins on the ventral wall of the canal was measured by using a flexible steel tape. The mean distance was 47.4 mm.; in 36% the distance was greater but in 42% it was less than the mean.

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[1] Aided by a grant from the United States Public Health Service.

Retrograde degeneration. Some evidence against the validity of Gudden's law in the spinal cord. A. M. LASSEK and J. H. PERRY, Department of Anatomy, Medical College of the State of South Carolina.

Gudden's law is defined as cellulipetal degeneration in the proximal end of a divided nerve. According to Brodal ('40), Gudden operated on animals 1 to 2 days after birth and found that all cells as well as the central and peripheral portions of the neuron disappear after a short post-operative course. In a modification of Gudden's method, Brodal ('40) produced lesions in the cerebellum of animals between 10 and 20 days of age and after 8 to 12 days post-operatively found practically total loss of the affected cells in the inferior olive of the medulla oblongata. He utilized a cellular stain only and believed his modification would probably be satisfactory in similar studies of other structures of the central nervous system. In a series of twenty-four kittens varying in age between 1 and 10 days, in which hemisections were made at the level of the middle or lower portion of the first cervical segment, we have been unable to notice loss of axons proximal to the lesion 8 to 12 days or longer. The axis-cylinders were stained with Windle's modification of Ranson's pyridine-silver method. The neurons of the cord found to be most suitable for study at this age were those of the fasciculus gracilis and cuneatus. These fibers arise peripherally from unipolar cells of the spinal ganglia but on entering the spinal cord they lose their neurolemma sheaths and assume the characteristics of fibers of the central nervous system. Our conclusions are formulated on the basis of general observations. Further detailed studies are being carried out.

The human pyramidal tract. IX. Effect of paralysis caused by cerebral tumors on axons of pyramid. A. M. LASSEK, Department of Anatomy, Medical College of the State of South Carolina.

The effect of paralysis on the axons of the pyramid caused by cerebral tumors was studied in a series of 119 cases. Suitable sections of medulla oblongatas selected from about 2,000 cases of brain disease were stained with Davenport's original silver nitrate method following formalin fixation which is specific for the axons. Sixty of the specimens were obtained through the cooperation of Dr. Joseph Globus of Mount Sinai Hospital; forty-nine cases were obtained through the cooperation of Dr. Abner Wolf of the Neurological Institute and ten cases were obtained through the cooperation of Dr. Charles Davison of Montefiori Hospital, all of New York city. All of the cases exhibited one or more of the classcial pyramidal tract signs but especially the pathological reflex of Babinski. Within the limitations of the material and method used in the investigation, there is some ground for the following conclusions: In cases of cerebral tumors, unilateral motor deficit, with one or more pyramidal tract signs, may occur with little or no loss of pyramidal tract axons. In this series, eight or 6.7% show complete destruction. Apparently, tumors located in any part of the cerebrum may produce motor difficulties with pyramidal tract signs. Tumor masses which caused complete loss of axons were widespread in extent involving more than one lobe of the cerebrum and usually subcortical structures. It is difficult, in this group of cases, to correlate the terminology employed in describing paralysis with the degree of pyramidal tract destruction.

A case of complete absence of the inferior vena cava. Homer B. LATIMER and Herbert H. VIRDEN, Department of Anatomy, University of Kansas.

This interesting case was found in the laboratory, in a white male cadaver, past middle life, well nourished, large and heavily muscled. Due to a recent fire, all information concerning this cadaver has been lost.

There was complete absence of the inferior vena cava and all blood from the lower half of the body was returned via the ascending lumbar veins and the azygos vein to a normal superior vena cava. The two ascending lumbar veins were apparently continuations of the common iliac veins. Each renal vein emptied into the common cardinal, or ascending lumbar, of that side. The left cardinal passed through the aortic hiatus, dorsal to the aorta and the right, passed through the medial lumbocostal arch. The left vein turned to the right just superior to the diaphragm and united with the right, and the resulting one large vein ascended, first a little to the right, next just in front of the vertebral column and then, at about the level of the base of the heart, turned to the right and anteriorly, and passing superior to the root of the lung, emptied into a normal superior vena cava just before it entered the heart.

The hepatic veins united and passing through the diaphragm, entered the right atrium in the position of a normal inferior vena cava. These veins appeared thin walled, much like a normal azygos vein, except for size.

The weights of the musculature in the fetal, newborn and adult cat. Homer B. LATIMER, Department of Anatomy, University of Kansas.

The skeletal muscles of the fetal cat increase from about 20, to a maximum of about 35% of the body weight, and to about 30% in the late fetal and newborn cats. In the adult cat, the musculature forms slightly less than half of the body weight. The percentage weight in the adults is at its maximum in the cats of medium size. The physiological condition of the adult cat makes a difference in the relative weight of the musculature.

The absolute weights plotted on fetal and newborn body length, form a curve concave superiorly. The weights of the muscles of the fetal and newborn cats plotted on body weight and all of the curves in the adults, for body weight and for body length, are straight line curves. Empirical formulae have been developed for all of these rectilinear curves by the method of least squares.

Correlations with body weight, body length and with the weights of many of the organs are all positive, although some are too low to be significant. They average + 0.65 in the males and + 0.55 in the females.

In the smallest fetuses, the musculature is very slightly heavier than the skeleton. This ratio increases to about 2.5 in the late fetuses and newborn cats and to 3.71 in the adult males and 3.85 in the adult females.

Phenylhydrazones and cholesterol crystals in the adrenal cortex of albino rats following repeated heavy dosages of insulin. John S. LATTA and George A. GOSTAS, Department of Anatomy, University of Nebraska.

A group of nine male albino rats received seven daily intraperitoneal injections of 80 units of either protamine zinc or crystallin insulin (courtesy of Eli Lilly Company). Adrenals were removed, weighed and prepared for study. These were compared with adrenals from a series of controls as follows: (1) no manipulation, (2) manipulated as experimental animals, with and without ether, (3) with repeated saline injections.

Treatment of adrenal sections with phenylhydrazine revealed a definite increase in phenylhydrazones (evidence of corticosterones, Bennett, '40) in the adrenal cortex of experimental animals particularly in the zona glomerulosa and outer part of the zona fasiculata.

Examination of sections with polarized light showed an apparent decrease in the number of birefringent crystals (suggested as possible cholesterol by Hoerr, '36, and Bennett, '40) in the adrenal cortex of insulinized animals.

We failed to find any relative increase in weight of adrenals of insulinized animals.

Further histochemical and cytological studies are in progress.

Action of androgens on the adrenal X-zone and kidney of the mouse. J. H. LEATHEM and H. D. SLOBODIEN, Department of Zoology, Rutgers University.

A single subcutaneous injection of 0.5 mg. of testosterone propionate caused the adrenal X-zone to disappear in 6 days in 22–24 day old female mice. A slight increase in kidney weight was observed. A marked increase in kidney weight can be produced with the 0.5 mg. total dose by injecting 0.05 mg. subcutaneously daily for 10 days. To compare the relative androgenicity of testosterone with methyl testosterone, the latter hormone was administered to mice in single subcutaneous injections of as much as 2 mg. but this dose failed to cause X-zone disappearance or kidney weight increase at the end of 6 days. If, however, mice received 0.5 mg. of methyl testosterone daily for 10 days then the adrenal glands were found to be devoid of an X-zone and an increase in kidney weight was noted. Using the kidney and adrenal as indicators of androgenic activity it is evident that testosterone propionate is at least five times more active than methyl testosterone under the conditions of the experiment. Furthermore the dosage of an androgen needed to cause X-zone disappearance can also increase kidney weight.

Hiatus canalis sacralis in American White and Negro males.[1] Gordon S. LETTERMAN and Mildred TROTTER, Departments of Surgery and Anatomy, Washington University.

The failure of the laminae of the fourth and fifth sacral vertebrae to meet in the mid-line creates an opening at the caudal end of the sacral canal known as its hiatus. In 553 male sacra (Terry Anatomical Collection) the mean length of the hiatus from base to apex is 22.5 mm. The apex is located at the lower third of the body of the fourth sacral vertebra in 32% of the bones; in one-half of the sacra the apex is at levels superior to this. At the apex of the hiatus the sacral canal has a mean antero-posterior diameter of 5.3 mm. The hiatus as well as the lumen of the sacral canal may be reduced or blocked by a dorsalward projection of the ventral wall, by a transverse fold in the posterior wall, or by a pronounced flattening of either wall in relation to the contour of the other.

Variations encountered which alter the bony prominences are: an overgrowth of bone which may obscure the hiatus and elongate the posterior wall of the sacral canal; sacral cornua may be absent or markedly flattened. The dorsal bony wall of the sacral canal exhibits a multiplicity of peculiarities — complete agenesis, laminar deficiencies, unilateral, bilateral, and seriational apertures.

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[1] Aided by a grant from the United States Public Health Service.

Evidence of placental mammotrophin. Wm. R. LYONS, Division of Anatomy, University of California.

Thirty-three rats were hypophysectomized and oöphorectomized on days 7–8 of their only pregnancy and injected subcutaneously, daily until the day before autopsy as follows: Group 1, sixteen rats, 1 µg. estrone plus 3–4 mg. progesterone; group 2, eleven rats, 3–4 mg. progesterone; group 3, three rats, 1 µg. estrone; group 4, three rats, nothing. In group 1, twelve had living young when sacrificed on days 21–23 of gestation, and two died on day 22 while trying to deliver term fetuses. All fourteen showed lobulo-alveolar mammary growth equivalent not to that of term, but to that of the middle third of pregnancy, and early signs of lactation. The two remaining rats had resorbed and showed only mammary ducts after 2 weeks of treatment. In group 2, ten showed varying amounts of placental residues (one rat had a single living fetus) when sacrificed on days 22–23. Five of these showed lobulo-alveolar mammary growth equal to that occurring in the first third of pregnancy, and the others showed only ducts with sparse alveolar budding. The remaining rat had completely resorbed and showed only ducts. Groups 3 and 4 were autopsied after 1 and 2 weeks respectively. They had completely resorbed and showed only mammary ducts with sparse alveolar budding.

Apparently the placenta secretes substances comparable to those of the anterior pituitary which (1) synergize estrone and progesterone in their stimulation of lobulo-alveolar mammary growth and (2) cause lactation.

The attachment of fibers of the musculus rectus abdominalis to the annulus umbilicalis in the human. J. E. MARKEE and J. A. SPAULDING, Department of Anatomy, Stanford University and Department of Anatomy, Duke University.

The relationship of the musculus rectus abdominalis to the annulus umbilicalis was studied in 153 human bodies. In about 56% a unilateral or bilateral bundle of muscle fibers terminates in a fibrous band which inserts into the umbilical ring. In about 29% such bundles occur bilaterally. In only about 2% is the muscular band located cephalad to the umbilicus. The width varies between 1 and 13 mm.; the mean being about 6 mm.

The existence of fibers of the musculus rectus abdominalis caudal to the inscriptiones tendineae at the umbilical level, and the lack of corresponding fibers cephalad to this inscription, apparently explains the attachment of this portion of the muscle into the annulus. When this inscription is slightly cephalad to the umbilicus the muscle fibers or their fibrous insertion partly encircle the umbilicus. The contraction of this portion of the rectus muscle might, by a sphincter like action, tend to protect against umbilical herniation. However when this inscription is caudad to the umbilicus, the fibers attach to the caudal aspect of the annulus and might tend to open the umbilical ring and hence predispose to umbilical herniation. When this inscription is cephalad to the umbilicus on one side of the midline and caudad on the other these muscular bands attach to the cephalic and caudal aspects of the annulus on their respective sides.

A quantitative study of the supraoptic nucleus of rat, dog, and man. Robert L. MERRICK, Department of Anatomy, University of Minnesota. (Introduced by A. T. RASMUSSEN.)

On the basis of topographical relation to the optic chiasm and tract, the nucleus was divided into three parts: dorsolateral (anterior), ventromedial (posterior), and dorsomedial (scattered dorsal cells). In rats, a fourth portion was recognized, the intraoptic.

The material was sectioned frontally at 10 micra and supraoptic neurons with definite nucleoli were counted. The means, standard deviations, and coefficients of variation were determined for the total nucleus of a side by a systematic study of 82 rat, 28 dog, and 28 human nuclei, using every sixth, twelfth, and twentieth section respectively. These values were: means — 8,419, 46,218, 67,747; standard deviations — 419, 2,632, 6,627; coefficients of variation — 4.9, 5.7, 9.8. These constants were also computed for the subdivisions of the nucleus. Histograms of the total nucleus indicate the distributions to be of a relatively normal type.

Multinucleolated and multinucleated cells were rare and caused no significant error in counting. No significant differences were found in the number of cells in regard to sex, side, or age within the range studied.

The variability of the separate parts of the nucleus was always considerably greater than that of the whole nucleus. The coefficients of variation of the whole nuclei of rats and dogs were low. This coefficient in humans, while higher, was only slightly more than that reported for brain weight and volume.

Effects of bilateral simultaneous damage of the striatum and striopallidum in the monkey. Fred A. METTLER, Dept. of Neurology, College of Physicians and Surgeons, Columbia University.

While no type or combination of frontal decortications ever produces an impulsion to climb, the addition of striatal damage to such lesions commonly evokes this phenomenon which, in the primate, is, apparently, the homologue of the feline "leaping phenomenon." The addition of striatal damage to bilateral removal of areas 6–12 also accelerates the preexisting ambulatory hyperkinesia. If the striatal damage is extensive, the pattern of simple ambulation and climbing becomes complicated by the animal's inability to avoid obstructions and pitfalls from which it is incapable of extricating itself. Thus, although the animal is more active than before operation, it spends much of its time in situations in which it is unable to progress. Overactivity of at least two physiologic mechanisms is involved in the impelled progression of such animals. These are the positive supporting reaction and myotatic reflex. Such animals give the impression of being unusually sensitive to proprioceptive stimuli. The range of movement is decreased and the limbs are held in relatively neutral positions. A positive visual following phenomenon is also seen.

If, in addition to the striatum, both pallida are significantly injured, the animal becomes relatively indifferent to proprioceptive stimuli and hyperkinesia and the tendency to retain superimposed positions replace ambulation. The speed of movement is decreased. With increasing pallidal damage the level of alertness decreases and somnolence is gradually attained.

Effects of bilateral, simultaneous ablation of area 8, area 9, area 12 and areas 8-12 from the simian cerebral cortex. Fred A. METTLER, Dept. of Neurology, College of Physicians and Surgeons, Columbia University.

Bilateral, simultaneous, restricted decortication of either area 8, 12 or the posterior orbital gyrus (Brodmann's area 11, Walker's area 13) does not influence general bodily activity. Simultaneous restricted decortication of both areas 8–12 results in a marked increase in general bodily activity as a result of over-reactivity of a complex type. Both agranular frontal areas must be intact for this situation to occur. Bilateral, simultaneous, restricted decortication of areas 9 or 6 produces an increase in general bodily activity. This is the result of the appearance of a stereotyped, sustained, ambulatory pattern which is, apparently, spontaneous. A similar situation is seen after bilateral decortication of areas 6–12 or if bilateral striatal damage is added to bilateral decortication of areas 8-12. Simultaneous bilateral removal of areas 8–12 produces an extensor and abductor response to plantar stimulation.

Variations of the cystic artery and biliary tract.[1] N. A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Similar orientation of the cystic artery to the common hepatic duct and cystic duct is of sufficient constancy to warrant the concept of a cystic triangle, the boundaries of which are: infero-laterally, cystic duct; medially, common hepatic duct; cephalad, cystic or right hepatic artery.

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[1] Aided by a grant from the American Philosophical Society.

Characteristics of cystic artery: (1) Prevailingly single dividing into a superficial (peritoneal) and deep (non-peritoneal) branch. (2) Origin; high, intermediate or low; when high, usually, from a terminal hepatic branch. (3) Most commonly from right hepatic to right of hepatic duct. (4) Common hepatic duct crossed by cystic artery, i.e., lodged between right hepatic (dorsal) and cystic (ventral) arteries. (5) Right hepatic and cystic entirely ventral to bile tract. (6) Often double, the superficial and deep branch having a separate origin: (a) both arising to right of hepatic duct; (b) deep arising to right, superficial to left of hepatic duct; (c) deep from right hepatic, superficial from left hepatic; (d) deep from right hepatic, superficial from gastroduodenal, retroduodenal, or accessory right hepatic from superior mesenteric. (7) Entirely from latter vessel.

Site of union of cystic duct with common hepatic is more frequently low than high; types are angular, parallel and spiral. Cystic duct may swerve to the posterior or left side of common hepatic, descend with it in a common connective tissue sheath for a 2–3 cm. before terminating. Accessory hepatics may join the cystic, common hepatic or its branches. Filamentous types may join the gall-bladder or common hepatic.

Variations of the hepatic arteries.[1] N. A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

Anomalies noted: (1) Separate origin of hepatic from aorta (first instance in 200 dissections). (2) Complete hepatic from superior mesenteric it giving off the right gastroepiploic and retroduodenal. (3) Middle colic from celiac. (4) Middle colic from gastroduodenal. (5) Left colic from dorsal pancreatic, branch of celiac. (6) Continuation of gastroduodenal as retroduodenal. (7) Celiac having as its first branch right hepatic with left gastric, trunk ending in left hepatic and splenic. (8) Accessory right hepatic from dorsal pancreatic. (9) Left hepatic from splenic. (10) Hepatic branches from left inferior phrenic.

Characteristics of right hepatic: (1) Seldom ventral to common hepatic duct. (2) Usually dorsal to portal vein when accessory. (3) Spiralled around the common hepatic duct, i.e., first ventral then dorsal to the latter. (4) Exhibiting a half or full circle twist to right of hepatic duct, curved portion giving off the cystic. (5) Looped around one section of a vertically split (3 cm.) common hepatic duct, i.e., passing through it. (6) Main supply of caudate lobe by a branch which after passing under portal vein ''crawls'' along caudate process giving off many short twigs. (7) An inferior lobular branch to a fissured area of the right lobe because of its proximity to the cystic may be mistaken for it.

Left hepatic: (1) Main supply to quadrate lobe. (2) Often anastomosed with left gastric. (3) Often double, accessory from left gastric.

Terminal hepatic branches varied from 13–65, those entering the caudate and quadrate lobes were prevailing short and fine.

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[1] Aided by a grant from the American Philosophical Society.

The retroduodenal and supraduodenal arteries.[1] N. A. MICHELS, Daniel Baugh Institute of Anatomy, Jefferson Medical College.

1. Retroduodenal (posterior superior pancreaticoduodenal). This large (1–3 mm.), frequently tortuous artery occurs in all bodies (75), usually as the first branch of the gastroduodenal. Coursing downward it passes anterior, then posterior to the common bile duct to be distributed in arcade fashion on the posterior surface of the head of the pancreas. From the arcade and its subdivisions (secondary, tertiary arcades) branches are supplied to all three parts of the posterior surface of the duodenum in a more copious manner than are distributed the anterior branches from the anterior arcade formed by the superior pancreatico-

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[1] Aided by a grant from the American Philosophical Society.

duodenal and inferior pancreaticoduodenal. The retroduodenal unites with a posterior branch of the inferior pancreaticoduodenal or joins the superior mesenteric or a jejunal branch independently. It gives fine anastomosing branches to the pancreas, ascending and descending branches to the common bile duct and in instances supplies the superficial cystic.

2. Supraduodenal artery of Wilkie. This small artery is distributed to the upper border, anterior and posterior surface of the first part of the duodenum. It usually arises from the gastroduodenal below the retroduodenal. Often it is not an independent artery, but a branch of the retroduodenal or right gastric. It gives branches to the pancreas and common bile duct. It is not an end artery (Wilkie) as it frequently anastomoses with the right gastric, superior pancreaticoduodenal, retroduodenal, and via a transverse branch with the splenic.

Bilateral dimensions of the vertebral column. Meryl MILES, Department of Anatomy, Washington University. (Introduced by Mildred TROTTER.)

Observations and measurements were made on seventy vertebral columns taken from cadavers of which sixty were without apparent lateral curvature and ten showed marked lateral curvature.

While the ligaments were still intact the intervertebral foramina were cleared of all tissue and measured. After the columns were skeletonized vertical measurements were taken at the sides of the vertebral bodies.

The data on sixty columns (vertebrae from T4 to L5 inclusive) were studied statistically according to age, race, and sex.

No significant difference was present between the mean heights of the intervertebral foramina of the two sides. The heights of the foramina increased from above downward except for those between T8 and 9, T9 and 10 and between L4 and 5 where decreases were shown.

Differences between the mean heights of the two sides of the vertebral bodies were insignificant except for that of T6 in the males where the right side was greater. The heights of the sides of the bodies increased from above downward as far as L5 which showed a decrease.

Of the ten columns which presented lateral curvature, five had their convexity to the left and five to the right. The heights of the foramina and the lateral heights of the adjacent bodies present significantly positive coefficients of correlation.

Numerical and regional distribution of taste buds on the tongue of the pigeon. Charles Allan MOORE and Rush ELLIOTT, Department of Zoology, Ohio University, Athens.

A study of the structure, number and distribution of taste buds on the tongue of a bird, the pigeon, Columba domestica, has been made. Buds were found on the lateral surfaces of the tongue craniad of the tongue fold, on the lateral, medial and ventral surfaces of the tongue wings, and on the dorsal and lateral surfaces of the soft portion of the tongue caudad of the tongue fold. The greatest concentration of buds was found on the dorsal portion of the tongue caudad of the tongue fold, with 70.7% of the total number of buds observed being found in this area.

The maximum number of buds present on any one tongue studied was found to be fifty-nine, with a minimum number of twenty-seven, and with an average of slightly less than thirty-seven for all tongues studied.

No definite pattern of distribution of buds was observed on any area except on the dorsal surface of the portion caudad of the tongue fold where they were generally more concentrated near the midline.

The buds were scattered through the epithelium and not found in papillae, as in mammals. The only papillae present were of a type resembling mammalian filiform papillae and these were found on the tongue fold and on the tongue wings, but none of these papillae possessed buds.

The effect of testosterone propionate on the tonus of the urinary bladder. S. Richard MUELLNER and James B. HAMILTON, Department of Urology, Beth Israel Hospital, Boston, and the Departments of Anatomy, Yale University and University of Missouri.

Cystometric studies designed to determine if the tonus of the bladder may be influenced by androgenic treatment were carried out in six middle-aged and old men and two women with a cystometer similar to that devised by Simons (1935, J. Urol., p. 493) and equipped with a device for "leveling." The intravesical pressures observed with the introduction of increasing amounts of fluid into the bladder were higher in the men following treatment with male hormones. There was an increase in the maximal intravesical pressure developed both before pain or discomfort was first noted and before the pain was sufficiently severe that the test was terminated. The volumes of fluid contained in the bladder at the first appearance of pain and at the onset of severe pain were similar to or slightly smaller than those that produced similar phenomena prior to medication. In most instances the intravesical pressure at which the first desire to void was experienced was increased upon androgenic treatment, but the volume of intravesical fluid at this point did not vary in a uniform manner.

This augmented tonus of the musculature of the bladder was in excess of the normal range of variability and could not be accounted for by technical procedures. Neither the increased tonus nor an apparent improvement in control of micturition are attributable to change in prostatic size or consistency.

Formation of suture bones in man. Charles R. NOBACK, Department of Microscopic Anatomy, University of Georgia.

Suture bones in man "appear to be fortuitous embryonic ossifications" . . . "appear to be due to the detachment of 'marginal islands' of ossification from the edges of the normal bone" deBeer, '37. This is the generally accepted explanation of suture bone formation.

During the early fetal period the rapidly proliferating membranous calvarial bones always have detached osseous islands variably arranged on their peripheral margins. Soon after their appearance these islands are apparently incorporated into the bones. They are infrequently present during the late fetal period. These islands were seldom observed to be associated with the facial bones and they were never observed to be associated with the basicranial bones. (Noback, Anat. Rec. '43, 87: 29.) These structures are readily demonstrated in situ by alizarin red S or silver nitrate stains. The frequency of the distribution of the islands corresponds with the location of the suture bones, which are generally observed in the sutures of the calvarium, infrequently observed in the facial sutures and rarely observed in the basicranial sutures.

The hypothesis is advanced that the formation of some, possibly most, suture bones is due to the persistence and enlargement of these bony islands. Thus in man suture bones may be interpreted as persisting normally present embryonic structures and not exclusively as fortuitous ossifications or detachments..

Neuronal disease associated with intracytoplasmic inclusion bodies resembling toxoplasm or rickettsia. James W. PAPEZ, Cornell University, Ithaca, N. Y.

A primary neuronal degeneration associated with intracytoplasmic inclusion bodies is demonstrated in human brains. By ordinary stains five stages are recognized: (1) hyperchromatic reaction with blocks of Nissl substance in periphery of cell, (2) shaggy, ragged state of Nissl substance with irregular cell outline, (3) chromophile cells with diffuse staining of Nissl substance, (4) cells with vacuoles, blisters, deformities and poverty of Nissl substance, (5) shadow cells, cell fragments with voiding of inclusion bodies. Inclusion bodies survive this stage, move into perivascular spaces.

Citric or hydrochloric acid followed by sodium carbonate and cresyl violet shows inclusion bodies. They are discrete, round, elongated objects which grow in colonies within cytoplasm; one half micron in diameter to 2 or 3 microns in length. Larval, medium and large forms occur. They contain an internal staining, granular material around a clearer center. A membrane surrounds individuals and holds them together. They divide by fission. As neurons degenerate, inclusion bodies are voided in masses and pass to blood vessels where they cause local damage. Many individuals separate and migrate in nervous tissue. Primary benign symbiosis and secondary neuronal disruption, voiding and vascular damage is suggested. Yellow pigment is formed when bodies degenerate in cells. They resemble toxoplasma, rickettsia and virus bodies of lymphogranuloma v. Some resemblance to Golgi apparatus and mitochrondria. They occur in encephalitis, thalamic disease, neuronitis and various motor disabilities.

The innervation of the extrinsic ocular muscles in the human fetus. Anthony A. PEARSON, Loyola University and Baylor University.

The main cell groups in the adult oculomotor nucleus are clearly shown in late fetal development. The constituent cells are small, medium-sized and large multipolar neurons. Three groups of small cells constitute the Edinger-Westphal nucleus. The medium-sized cells form an unpaired nucleus in the midline. The large multipolar neurons form the lateral nucleus which is divided into dorsal and ventral parts. The majority of the neurons in the lateral nucleus send their axons into the oculomotor nerve of the same side. The axons of many neurons in the caudal half of the oculomotor nucleus cross the midline and enter the oculomotor nerve of the opposite side. The processes of the median nucleus and the Edinger-Westphal nucleus are much more difficult to follow.

Cells of the mesencephalic V type in relation to the oculomotor and the trochlear nerves. A few of these cells occur in the nuclei of these nerves and along their course. Certain cells of the mesencephalic nucleus at the levels of the oculomotor and trochlear nuclei send their processes toward these nerves.

It seems unlikely that cells of the mesencephalic nucleus contribute to the abducens nerve. Nerve cells can be demonstrated along the course of the abducens nerve in certain human fetuses. These cells may be the source of proprioceptive fibers within this nerve.

Experiments on the decussation and course of Mauthner's fibers in Amblystoma punctatum. Jean PIATT, Department of Anatomy, University of Pennsylvania.

Grafts of the Mauthner's cell region from embryos of stage 21 were substituted for the same region of the medulla in older host embryos, stage 34. Other operations consisted in transplanting entire medullae from stage 25 to the flank region of stage 31, with the A-P axis of the graft both harmonic and reversed. The first set of experiments (substitution) were designed to test the ability of the axon from the younger (donor) Mauthner's cell to cross its ipsilateral fasciculus longitudinalis medialis, on the assumption that the latter tract had grown beyond the decussation level before the donor fiber had developed enough to reach the midline. The second set of experiments (isolation) were designed to test the ability of Mauthner's fiber to grow toward its original posterior pole in the absence of longitudinal projection tracts.

The substitution experiments showed that Mauthner's fiber would still grow across the midline after the host fasciculus longitudinalis medialis had presumably already developed past the decussation level. The isolation experiments showed that Mauthner's fiber would always grow toward the original posterior end of the grafted medulla without the help of longitudinal projection tracts, and regardless of whether the A-P axis was harmonic or reversed. These results are interpreted as support for the thesis that the fasciculus longitudinalis medialis is not a necessary guiding agent in directing growth and decussation of Mauthner's fiber.

The human platelet. Paul RALPH, Department of Medicine, Ohio State University. (Introduced by C. A. DOAN.)

Human platelets are round (83.5%) and ovate (14.5%) discs. When studied in darkfield with the aid of a new platelet preservative (.01% OsO_4 in normal saline) the average diameter is 3.9 μ, extremes 2.7 to 4.47 × 6.8 μ (ovate forms). A peripheral sheath of ectoplasm surrounds a central group of granules composed of two types of mitochondria, differentiable by their supravital staining reactions present in all platelets and a larger specific alcohol-soluble granule present in 56.5%. The mitochondria survive fixation as the "central body."

The normal platelet begins to degenerate 5 to 10 seconds after extravasation and becomes sticky, the first of an orderly series of degenerative events. This cohesiveness, by which the platelet performs its function in hemostasis, is caused by the action of serum tryptase since heparin inhibits this enzyme and prevents the platelet-stickiness although permitting their otherwise normal degeneration. The absence of cohesiveness in hemophiliac platelets reflects the low serum tryptase level. There was no evidence that the platelet or its degeneration contribute to fibrinogenolysis. The use of OsO_4 preservative and darkfield illumination are necessary for a study of normal morphology in normal blood since previously employed reagents preserve only a recognizable remnant of such platelets.

Dameshek's method ('36) proved to be the best for the enumeration of platelets. No objective method of determining the number per c. mm. of blood was found. Since in consecutive drops of blood from the same puncture counts varied as much as 800.000 per c. mm. differences between single counts within the normal extremes (200,000–1,500,000) have questionable significance.

Observations upon normal adult human erythrocytes. Paul RALPH, Department of Medicine, Ohio State University. (Introduced by C. A. DOAN.)

Under darkfield illumination a very few immature red cells (reticulocytes) showed small rods which proved to be mitochondria. With low concentrations of several supravital stains some reticulocytes develop, de novo, stain-containing vacuoles which in darkfield differ in color from the reticulum, also produced de novo. Both may exist in the same cell but are not thought to be variant expressions of the same interacting factors. The Isaacs granule ('25) is an artifact since it cannot be seen in freshly drawn blood. Five stages of maturity are found in the erythrocytes of normal circulating blood, those displaying; 1. Mitochondria, vacuome and reticulum; 2. Vacuome and reticulum; 3. Reticulum; 4. Isaacs granule and 5. No internal structure (mature erythrocytes).

In extravasated blood the erythrocyte membrane swiftly alters so that it adheres to the glass of coverslip and slide, platelets and other red cells. At the point of adherence the cell membrane is often drawn out in a fine, long thread which can be seen only in darkfield. This cohesiveness may be a factor in hemostasis but is not a specific erythrocyte-platelet conjugation (Pennell '43) and does not contribute to fibrinogenolysis. This alteration is apparently caused by the action of serum tryptase since heparin completely suppresses it and also inhibits the enzyme. The absence of this phenomenon in hemophiliac blood reflects a lowered serum tryptase level.

An experimental technique utilizing the "lymph sac" of the thyroid gland (cat). A. J. RAMSEY, The Daniel Baugh Institute of Anatomy, Jefferson Meical College.

The lymph-space of the thyroid gland of the domestic cat presents a unique opportunity for the experimentalist to study the activities of the gland.

Using ether anesthesia, a portion of the right thyroid lobe is removed (to be used as a control) and placed in fixing fluid. A ligature is placed around the caudal end of the left lobe, a small drop of dilute carbon ink is injected into this lobe, and any lymphatic vessels carrying the ink from the gland are secured and ligated. The experimental material is introduced into the left lobe and at the end of the experiment samples of tissue are taken from both lobes. The tissue from the right lobe is used as a second control while that from the left side demonstrates the effects of the experimental material.

This technique presents a method for obtaining, from the same animal, experimental and control data. Initial experimental studies utilizing this technique have shown it to possess marked advantages, including: (1) speed and depth of the reaction are increased; (2) the use of much smaller amounts of the experimental agent is possible; (3) the experimental material does not reach other endocrine structures in significant amounts; (4) the right lobe of the gland may be used as a control, the left lobe as the experimental field.

Growth of the human hypophysis during childhood. A volumetric study. A. T. RASMUSSEN, Department of Anatomy, University of Minnesota.

Volumetric analysis, based on serial sections of 122 selected hypophyses from cases of sudden (usually accidental) death, varying in age from birth to 19 years, shows that the gland (without infundibulum, pars tuberalis, meninges and capsule — which diminishes the crude weight by about 20%) increases from about 120 mg. to 500 mg. in males and from about 112 mg. to 600 mg. in females. Lines drawn by inspection of double weighted medians (based on age and weight) at 7 intervals are essentially straight with a tendency for pars distalis and the whole gland to curve upward slightly commencing at about 10 to 12 years in girls. The relative volumes of the lobes remain practically unchanged — pars distalis representing 77%, processus infundibuli 21%, and pars intermedia 2%, which are adult proportions. Pars distalis tends to form a larger proportion of the organ in females than in males and the parenchyma of pars intermedia of both sexes constitutes a slightly diminishing per cent of the gland with increasing age during the period considered. The amount of colloid amenable to evaluation is too variable to be plotted beyond scatter diagrams; but there are indications that it fluctuates in a manner to suggest cyclic activity. The coefficient of variation within each age group is of the order encountered in the adult. Pars intermedia is the most variable portion.

Concerning certain projection fibers of the superior olivary complex. G. L. RASMUSSEN, Department of Anatomy, University of Buffalo. (Introduced by Oliver P. JONES.)

Small lesions placed within and about the superior olivary complex of several cats reveal after the Marchi method, the following noteworthy findings.

The vast majority of the so-called olivary peduncle fibers observed streaming dorsally from the region of the accessory olivary segment toward the 6th nucleus actually leave the peduncle sharply at right angles some distance ventral to the abducens nucleus. (The remainder continue beyond the 6th nucleus to become associated with the heterolateral cochlea (G. L. Rasmussen, '42)). They cross obliquely forward through the reticular formation as widely scattered fibers but converge upon entering Monakow's field (area occupied by the stria medullaris acoustica) of the opposite side. These fibers are numerous but not conspicuous due to their small caliber and manner of dispersion. There is a corresponding homolateral bundle of about half as many fibers which also leaves the accessory segment and passes directly into Monakow's area overlying this nucleus. Both fiber groups terminate chiefly in the medial portion of the dorsal lateral lemniscus nucleus. The remaining few fibers end at the base of the inferior colliculus.

Furthermore, contrary to prevalent teaching but in accord with Monakow's (1890) contention, the Marchi experiments clearly show that practically all the olivary complex fibers stand in relation to nuclei of the lateral lemniscus. Only a few fibers reach the inferior colliculus and none terminate in the medial geniculate body.

The relative resistance of nerve fibers and their nutrient vessels to trauma. G. I. RASMUSSEN and F. KREDEL, Departments of Anatomy and Surgery, Medical College of the State of South Carolina and Department of Anatomy, University of Buffalo. (Introduced by J. Graham EDWARDS.)

Previous experiments (Authors, '43) with traction-produced, transient nerve blocks indicated that nutrient vessels may be less resistant to trauma than nerve fibers and that periaxial changes in the latter are the result of circulatory disturbances. To test this further, miniature blood pressure cuffs 1 cm. wide were applied directly to dogs' sciatic nerves. Double cuffs separated by an interval of 1–2 cm. in 10 and single cuffs in 4 experiments were fitted to the nerves. These were inflated to pressures varying from 140–300 cm. Hg for periods of ½ to 1½ hours. Animals were sacrificed 7–10 days post-operative and portions of each nerve were treated by Marchi, silver and Masson technics.

It was observed that occlusion of intraneural venules, epineural veins and occasionally arterioles present at the sites of compression interrupts normal circulation with resulting venous stasis followed by edema. The main foci of edema (7th day) and associated mesenchymal hyperplasia with thinning of myelin sheaths are not located at sites of initial trauma and ischemia, but proximal and distal to upper and lower cuffs or, in single-cuff experiments, beyond its margins. The more normal portion becomes progressively involved after the first week.

These results suggest that the thinning of myelin sheaths or demyelinating process (without obligatory terminal Wallerian degeneration) as seen in some types of transient paralysis is primarily due to vascular disturbances.

Formation of commissures in surgically altered brains of chick embryos. Ruth RHINES, Department of Anatomy and Institute of Neurology, Northwestern University. (Introduced by W. F. WINDLE.)

Observations were made on altered commissure formation in mesencephalic and metencephalic chick embryos from which the rostral end of the brain had been removed at 40 hours. Another type of operation produced a gap in the brain stem by excision of the metencephalon. Effects observed in pyridine-silver preparations indicate that conditions reported for crossed Mauthner's fibers in Fundulus embryos do not hold for commissures in the chick. Ventral longitudinal tracts do not induce growing axons to cross the midline, for commissures were noted in the absence of any longitudial tracts, agreeing with observations in very young unoperated cat and chick embryos that commissural axons entered the floor plate before any longitudinal tracts formed.

In the chick the shape of the neural tube appeared to be a factor influencing the course of growing axons. In the rounded ends of healed brains, fibers of local origin ran transversely and fibers ascending from other levels reached the end of the brain where they followed the curve of the rostral wall. More caudally, where a ventricle was present, the thick ventral part of the neural tube was the site of a radially arranged commissure. The normally ipsilateral diencephalic medial longitudinal fasciculus crossed the midline near the blind ends of interrupted brains. At other levels pockets in the floor plate deflected crossing fibers, forming a midline longitudinal tract.

Arrested development of turtle embryos. Paul L. RISLEY, Department of Zoology, The State University of Iowa.

At egg-laying, the turtle embryo is invariably in stages of early gastrulation. Adult females collected in season are forced to retain the eggs in utero for periods of 2 to 6 weeks beyond the usual time of deposition. Embryos examined at autopsy show no development beyond the stage at which they are normally laid. Among several hundred specimens observed, only one developed as far as the sixteen somite stage in 4 weeks. Exceptional specimens show weak neural plate formation and elongation of the embryonic disc. The blastoderm spreads slowly, and the area pellucida increases greatly.

With care in incubation, 50 to 75% of the embryos may be expected to continue their development normally. No consistent malformations occur subsequently, although microcephaly is sometimes observed. Tendencies toward twinning or axial duplication have not been found.

Cessation of gastrulation and further differentiation under controlled anaerobiosis for short periods has been noted by Brachet ('34) for frog embryos, but is not evident in toad development. By analogy with the frog it is probable that the turtle uterus is an anaerobic chamber. The stability of turtle embryos under these conditions is remarkable, even more so than the frog. The nature of the material and simplicity of the method provides a unique opportunity for physiological studies of early developmental processes.

Nature of the early somatic movements in Fundulus heteroclitus. Charles H. SAWYER, Department of Anatomy, Duke University. (Introduced by J. E. MARKEE.)

In embryos of Fundulus heteroclitus spontaneous movements begin after morphological innervation of the trunk musculature. However there is practically no cholinesterase (ChE) present at this time and esserine, a ChE inhibitor, does not affect the movements. They are therefore interpreted as myogenic, perhaps initiated by an unbalanced ionic or osmotic environment acting directly on the muscles.

Shortly later in development, heavy compression of the fourth ventricle stimulates a flexure response; light tactile stimuli are ineffective. By this stage the esterase level has risen significantly and the reaction is potentiated by eserine. Hence the response is considered neurogenic.

The capacity for truly reflexogenic activity, the ability to respond to light tactile stimuli, is accompanied by a further increase in ChE content and eserine potentiation; flutter and swimming reactions are attended by an ever increasing enzyme level. In the later stages eserine leads to an opisthotonic quiver swimming response and finally to a tetanic bend-reaction, retarded growth, paralysis and death. The effective concentration of the drug is at most only one-tenth that employed in Amblystoma, a fact which may be related to the much greater acetylcholine-cholinesterase metabolism in Fundulus.

The vascularity of neuropil. Ernst SCHARRER, Department of Anatomy, Western Reserve University.

The rich vascularity of some neuropils has been attributed to the presence of many synapses. This explanation cannot be generalized, however, because there are also poorly vascularized neuropils. Extremes illustrating this discrepancy are represented in the teleost brain by the molecular layer of the cerebellum and the neuropil of the nucleus rotundus. While these two neuropils are comparable with respect to the number of synapses, they show a marked contrast in the number of mitochondria. In the highly vascular neuropil of the nucleus rotundus, mitochondria are densely packed, whereas in the poorly vascularized molecular layer of the cerebellum they are scarce. This correlation is not restricted to the fishes, but applies to neuropils of other vertebrates as well. Since respiratory enzymes have been demonstrated in mitochondria (Lazarow and Barron '41) the difference in the number of mitochondria might well express dissimilarities in the metabolic rates of the synapses of different neuropils. It is suggested, therefore, that the vascularity of a neuropil is correlated not so much with the number as with the metabolic requirements of the synapses.

A comparison of cerebral vascular patterns in vertebrates and invertebrates. Ernst SCHARRER, Department of Anatomy, Western Reserve University.

The vertebrate brain may be vascularized either by single arteries and veins that anastomose in a capillary network, or by paired blood vessels that end in capillary loops. The first type has been found in all fishes studied so far, in a number of amphibians and reptiles, in all birds, and in the placental mammals. The second kind occurs in certain amphibians and reptiles, and in the marsupials. There are also invertebrates whose central nervous system is supplied by blood vessels. Havet ('16) mentions intraganglionic blood vessels in the earthworm, and Cajal ('29) describes them in the squid. The vascular patterns in the central nervous system of these invertebrates have been compared with those of vertebrates. In the earthworm the blood vessels are of the marsupial type: they are paired and end in hairpin-like loops that do not anastomose with each other. By contrast the arteries and veins of the squid brain are arranged in the same way as in placental mammals: the blood vessels are single and form a network of anastomosing capillaries. The occurrence of both, capillary reticulum and terminal loops, in the invertebrates further complicates the problem of the phylogenetic relationship between these two types of cerebral vascularization.

Thyroidectomy in the rat on the first day of life. Robert Oliver SCOW, Division of Anatomy, University of California. (Introduced by H. M. EVANS.)

The growth and maturation of the rat is greatly retarded when the thyroid gland is removed at birth. This was first shown by Salmon, whose animals' longevity was 60 days. 440 newborn rats of the Long-Evans strain were thyroidectomized under refrigeration anesthesia. The methods of determining completeness of thyroidectomy included the radioautograph method, skeletal development, body weight gain, and oxygen consumption. They were maintained before and after weaning (at 28 days) at 25° to 29°C. and were fed nightly with moistened McCollum's Diet 1. Dry diet was available at all times. 25 completely thyroidectomized rats were sacrificed at 56–141 days of age. Their characteristics during the first 25 days closely corresponded to those reported by Salmon. The development of the external ear was retarded. Ten rats thyroidectomized at birth had the following typical characteristics after 45 days of age: Slow continuous increases in body weight (0.3 grams daily) and of skeletal dimensions, delayed appearance of epiphyses (skeletal age of 18 days at chronological age of 60 days), reduced oxygen consumption (–20%), markedly retarded hair transformation from the infant to the adult type (occurring at 110 days), and delayed gonadal development. There was no apparent increase in susceptibility to intestinal disorders, including meteorism, and no symptom of tentany. The thyroidectomized rats displayed a hearty appetite and normal activity.

Effect of growth hormone in rats thyroidectomized at birth. Robert Oliver SCOW and Walther MARX, Division of Anatomy, University of California and Institute of Experimental Biology. (Introduced by H. M. EVANS.)

The growth rate of rats completely thyroidectomized at birth was stimulated by injections of a purified preparation of growth hormone. The method of thyroidectomy and the determination of its completeness is described in the preceding paper. The growth hormone used was a cysteine treated globulin fraction and was practically free of other known pituitary hormones. Eleven rats thyroidectomized at birth were injected with 1 mg. of growth hormone daily for 23 days, commencing at 30 days of age. The injected animals gained 35 grams (100% increase) whereas seven uninjected controls gained 7 grams (17% increase). The length of the tibia of the injected animals increased 6.6 mm. in comparison to 4.8 mm. in the controls. The growth response could be elicited in animals 51 to 58 days post-operative. These findings are in contrast to those of Salmon. The growth response was greater in incompletely thyroidectomized rats than in the completely thyroidectomized rats. During the 22 days following the cessation of the injection, however, there was no increase in body weight or skeletal size. During the period of injections, no acceleration was observed in the appearance of the epiphyses, transformation of hair nor vaginal openings.

Retarding effect of brief refrigeration upon skeletal development in the newborn rat. Robert Oliver SCOW, Division of Anatomy, University of California. (Introduced by H. M. EVANS.)

A startling retardation has been observed in the growth and differentiation of the skeletal parts of the limbs of newborn rats when the limbs came in contact with extremely cold surfaces. This phenomenon was observed during the use of a type of refrigeration anesthesia in which the rat was placed for 15 minutes in a glass beaker (internal temperature, –5° to –10°C.) which was partially submerged in a mixture of ice cubes and brine. (The resulting anesthesia lasted for 8 to 15 minutes.) After coming in contact with the beaker's surface, the growth and ossification of the affected vertebrae and feet were either nullified or retarded. The resulting valgus deformity of the feet appeared to be the result of strain exerted upon the unossified limbs by the weight of the body and the action of the muscles. The growth of the soft tissues, grossly, did not seem to be affected. More drastic retardation of ossification was produced in the specific parts of limbs held against a surface colder than that mentioned above. Their body weight at 31 days of age was not appreciably below normal. When refrigeration anesthesia is employed for newborn rats, it would appear that precautions should be taken to prevent contact between the animal and the surfaces of the refrigeration apparatus.

Electro-anatomical studies on a tactile system in the cerebellum of monkey (Macaca mulatta). Ray S. SNIDER and Averall STOWELL, Department of Physiology, The Johns Hopkins University.

Discrete tactile stimuli produce well localized potential waves in certain regions of the cerebellar cortex of the monkey under chloralosane-nembutal (75%–25%) anesthesia. By using a 3-stage resistance-capacity coupled amplifier and a cathode ray oscillograph, it has been possible to record these localized evoked cerebellar responses and to relate them (in the form of an anatomical map) to tactile regions of different parts of the body.

Two cerebellar areas receive these tactile projections: (a) the ipsilateral anterior lobe and lobulus simplex and (b) the ipsilateral and contralateral paramedian lobules. Apparently, the entire tactile surface of the body is represented in an orderly sequence in each cerebellar area. This observation on the monkey confirms earlier work on the cat (Snider and Stowell, '42). Further, in the rat Dow and Anderson ('42) have evoked cerebellar action potentials by tactile stimulation.

Occasional responses are found ipsilaterally in the medial portions of Crus I and Crus II. The latency of the average response in the anterior lobe is 20 msec.; in the paramedian lobule it is 45 msec. Work is in progress to determine the pathway or pathways which carry these impulses.

The effect of asphyxia on the hemoglobin concentration, red blood volume and plasma protein concentration of the newborn. Franklin F. SNYDER, Departments of Anatomy and Obstetrics, Harvard University.

In the course of observations on the tolerance to oxygen want of newborn rabbits, it was noted that the heart continued to beat long after cessation of respiration. In view of the striking resistance of the newborn to respiratory failure when deprived of oxygen as shown by the finding that respiratory movements continue one-half hour in rabbits born at term, 32 days, and may persist an hour or longer in animals delivered prematurely at 29 days, the question arises as to what circulatory changes occur in the course of asphyxia. Evidence of shock was sought by determination of the hemoglobin concentration, red blood cell volume, and plasma protein concentration. Blood samples of 0.2 cc. were obtained from the jugular vein before asphyxia and from heart puncture at the cessation of respiration. A series of 20 animals was obtained from 14 litters. Four fetuses were delivered prematurely near the onset of viability, 29 days. Blood samples obtained after cessation of respiration showed relatively little change in hemoglobin concentration, hematocrit determinations, or plasma protein concentration despite the prolonged period of oxygen deprivation.

The lateral geniculate complex of Macaca mulata. Othmar SOLNITZKY and B. Lionel TRUSCOTT, Georgetown University Brain Research Institute.

The lateral geniculate body is composed of six cellular laminae, everted and arranged concentrically about the hilus. The ventral two laminae contain larger (27.2 μ × 15.5 μ) and smaller cells (18.9 μ × 13.6 μ). The dorsal four laminae also contain two types of cells: medium-sized (17.4 μ × 11.1 μ) and smaller elements (10.9 μ × 8.9 μ). In addition to these six cellular laminae, there is present another slender lamina of smalll cells within the optic tract and ventral to lamina 1. It is significantly shorter than any of the other cellular laminae.

The hilus begins as a shallow groove a short distance caudal to the oral pole of the lateral geniculate body; it gradually deepens near the middle third of the lateral geniculate and growing shallower again, ends on the posterior aspect of the lateral geniculate.

The nucleus pregeniculatus is clearly subdivided into a medial and a lateral part. The medial part contains a richer neuropil and cells of two categories: larger (23.6 μ × 16.3 μ) and smaller elements (15.5 μ × 17.5 μ). The lateral part appears gelatinous due to the paucity of neuropil; its cells are smaller (17.5 μ × 13.6 μ to 9.7 μ × 13.6 μ) and more compactly arranged. The medial part also has a greater anteroposterior and dorsoventral extent than the lateral part.

The nucleus intergeniculatus is a fairly large mass, divisible into a larger ventral region of small cells (12.9 μ × 11.6 μ) and a smaller dorsal of larger cells (20.5 μ × 14.5 μ).

The regeneration of denervated lateral-line organs. Carl Caskey SPEIDEL, School of Anatomy, University of Virginia.

Regeneration of lateral-line organs has been watched in the frog tadpole's tail following amputation of the distal portion. Lateral-line organs near the cut edge give rise to placode material which rapidly grows into the regenerating tail tip zone. Accompanying the placode is the regenerating lateral-line nerve. Typical young lateral-line organs with characteristic sensory hair cells are differentiated within a few days.

Lateral-line nerve section, either before, after, or at the same time, has been combined with tail tip amputation. Some animals were subjected to multiple successive nerve section on one or both sides. Lateral-line organ regeneration, however, proceeded quite normally. Many individual case histories have been recorded which demonstrate clearly that all steps in the regenerative processes take place, including the differentiation of sensory hair cells, in spite of the absence of the lateral-line nerve. Similar experiments on Triturus larvae have given similar results.

These findings support the view that regeneration of special sensory organs may go on quite independently of their specific nerve supply. They militate against the view that special sensory organs are induced by the presence of their specific nerve supply.

Illustrative ciné-photomicrographs have been obtained.

The trophic influence of specific nerve supply on special sensory organs, as revealed by prolonged survival of denervated lateral-line organs. Carl Caskey **SPEIDEL**, School of Anatomy, University of Virginia.

Many investigators believe that special sensory organs, deprived of their specific nerve supply, degenerate within a few weeks. Stone ('40), however, finds that transplanted taste organs may survive and grow without innervation for nearly 2 months. He concludes that nerve supply is not essential to the maintenance of their structural integrity.

I have watched many individual clusters of denervated lateral-line organs in frog tadpoles for from 3 to 12 months. These observations reveal that (1) some denervated organs may survive for 12 months, (2) while there is little difference during the first few months between denervated organs and innervated controls, there is conspicuous difference after longer periods. One tadpole, for example, a month after lateral-line nerve section on both sides, had a zone of denervated organs on the right side (23 organs in 8 clusters), and a corresponding zone of re-innervated organs on the left side (28 organs in 9 clusters). Two months later there was little change. Nine months after the nerve section, however, the denervated right side had only 8 organs surviving, a decrease of 15. In sharp contrast the re-innervated left side had 49 organs, an increase of 21. Thus, lateral-line nerves exert a clearcut trophic influence on lateral-line organs which markedly affects their proper maintenance and growth over a prolonged period.

Illustrative ciné-photomicrographs have been obtained.

The effects of castration and cryptorchidization on the blood picture of the golden hamster. Kathryn **STEIN** and Barbara **JACOBSEN**, Department of Zoology, Mount Holyoke College.

The number of erythrocytes in the blood of male hamsters increased from 5.4 million per cu. mm. at 21 days of age to 8.8 million at 40 to 45 days, and continued at this level up to 226 days, the age of the oldest animals counted. Castration at 37 or at 80 days caused a lowering of the count to 6.4 million. a level which was attained more quickly in the adult than in the young animal. Decreases in the counts also occurred in the cryptorchidized animals and in a control subjected to similar operative procedure. The decrease, however, was less than that following castration and the counts gradually returned to normal. The total hemoglobin was estimated at about 20.6 grams per 100 cc. in the sexually mature animal, slightly lower values being found prior to this time. The cells in young animals had larger diameters than those of later age groups. Reticulocyte counts were variable and consistent differences were not found in the small group of animals studied. The total hemoglobin decreased following castration but there was no significant variation in cell diameters. Differential white counts were made but no significant variations were observed.

Factors in nasopharyngeal occlusion. Leon H. **STRONG**, Department of Anatomy, University of Michigan.

The most superior fibers of the constrictor pharyngis superior have a relatively heavy fascicle from the palatine aponeurosis. This passes lateral to and in contact with the levator veli palatini. A component of its force is medial. The levator contacts, laterally, the tip of the auditory tubal cartilage. The pharyngobasilar fascia pendent from the lower border of the tubal cartilage, turns acutely lateral, just below the tip, to ensheath the levator. The border formed by this turn projects medially. This border, and aponeurosis immediately continuous with it, is studded with and thickened by glands. This aponeurotic border extends from the tubal cartilaginous tip to the center of the sulcus (veloconstrictor), between the velum and the dorsal pharyngeal wall, forming the skeleton of the salpingopharyngeal fold. Up this border, from the underlying palatopharyngeus muscle mass, course fibers which usually reach the cartilage in one or more small bundles called the salpingopharyngeus.

The constrictor ridge (Passavant's) contacts the elevated and thickened uvular region. The uvuloconstrictor bridge, thus formed, occludes the nasopharynx from below, in approximately its middle two fourths, only. The combined sphincteric contraction of the superior constrictor (palatine slip especially), levator, palatopharyngeus, and salpingopharyngeus forces the structures composing the salpingopharyngeal fold medially. The salpingopharyngeal fold contacts the uvuloconstrictor bridge, completing occlusion. The specialized fat pad situated between the tensor and levator implies these movements, as well.

Some functions of palatine aponeurotic force components. Leon H. STRONG, Department of Anatomy, University of Michigan.

The palatine aponeurosis may be considered as the tendon of the tensor veli palatini. From the palatine bone to the hamular level it is thick. The inferodorsal continuation of this aponeurosis forms a thin sheet for the attachment of the velar musculature of both sides. It is fused laterally, along a line extending from the tip of the hamulus to the lower velar limit, with the pharyngobasilar fascia which invests the superior constrictor. This fusion forms a veloconstrictor sulcus. The sulcus is occupied mostly by the salpingopharyngeal fold. Medially, the sheet follows the curvature of the velum to the midline somewhat above the uvula, where it is continuous with its fellow half.

This sheet's plane, radiating from the hamulus is inferodorsomedial; the force components pull superoventrolateral, maintaining a tension which keeps the velum away from the dorsal pharyngeal wall. That wall slopes dorsally as well as superiorly, to gain the pharyngeal tubercle, so that the higher the level in the veloconstrictor sulcus, the greater the distance of the velum from the dorsal wall.

Hence, a uvuloconstrictor bridge cannot close the lateral port inherent in this architecture. The salpingopharyngeal fold is uniquely constructed so that forces move it medially to contact the uvuloconstrictor bridge, which completes occlusion.

Lack of assessing these facts leaves the functional picture of the velum incomplete.

On studying the pulmonary alveolus in the living. Robert J. TERRY and William L. SIMPSON, Department of Anatomy, Washington University.

Experiments to explore the alveolus during respiration have been in progress since the invention of a thoracic window by one of us in 1939. Sufficient illumination with control of temperature has been a serious difficulty. Light transmitted by a fused quartz rod introduced via the mediastinum, and direct light have both been employed. The question of interest at present concerns possible form and size changes of the alveolus during the respiratory cycle. The window is placed in the third right intercostal space of an adult cat under nembutal anaesthesia. The microscope, balanced on ball-bearing pivots, is connected with the window and remains in focus during chest movements. In making motion pictures of the alveoli against the thoracic window, light from an arc lamp is directed on the surface of the lung during the period of one cycle and then shut off. After an interval of several minutes another exposure is made. Significant rises of temperature are thus avoided. The respiratory curve is recorded simultaneously with the photographing. Every fourth frame of the 48 (circa) in the cycle has been projected and photographed on a grid, giving data for study of whatever changes in shape and size a given alveolus may undergo and of their correlation with phases of the curve. To the eye changes seem slight, but conclusion rests on measurements now in progress.

A re-study of the fasciae involved in penile urinary extravasation. Charles E. TOBIN and John A. BENJAMIN, Departments of Anatomy and Surgery (Division of Urology), The University of Rochester.

Studies were made of the perineal, scrotal, penile and ventral abdominal fasciae of one cadaver from an extravasation case and of nine normal cadavers. In four of the latter, extravasation was simulated by separating the fascial layers with compressed air introduced beneath Scarpa's fascia, and by injecting liquid latex into the penile urethra, perforated previously by a scalpel in a Young's urethrascope.

After traversing the urethral wall, the corpus spongiosum urethrae and its tunica albuginea, extravasation may be limited by Buck's fascia which forms three compartments around the vascular components and muscles of the penis, in the urogenital triangle, and extends in the form of a "figure of 8" along the shaft of the penis to enclose the corpora cavernosa penis and corpus spongiosum urethrae in separate compartments. This fascia is continuous with the suspensory ligament.

If Buck's fascia is perforated, further spread is found between Buck's and Colles' fascia in the perineum; dartos and external spermatic fasciae in the scrotum; Buck's and dartos fasciae in the penis; Scarpa's fascia and the aponeurosis of the external oblique muscle.

This study verifies the continuation of Camper's and Scarpa's fasciae as the dartos fascia of the penis and scrotum and as the subcutaneous adipose tissue and Colles' fascia of the perineum, and confirms Buck's description (1848) of the penile fascia, which is omitted in many texts.

Local reactions to subcutaneous injections of cholesterol. Edna H. TOMPKINS, Department of Anatomy, Vanderbilt University.

Mice were injected subcutaneously with colloidal suspensions of cholesterol in glucose. Films of connective tissue were examined supravitally, and subsequently with polarized light. Other films were examined with Sudan IV, nile blue sulphate, osmic acid gas, the Schultz technique for cholesterol and Leulier and Revol's technique with digitonin. Statements of time refer to intervals following injections.

Acicular crystals of cholesterol adhered to the tissue clasmatocytes early, and occurred free up to 36 hours. Neutrophiles appeared in 3 hours, and monocytes in 9. The former took no part in disposal of the cholesterol and disappeared within 60 hours. The monocytes rapidly developed into macrophages and acquired crystals on their surfaces.

The transformations of the macrophages and of the tissue clasmatocytes were essentially similar, except for the timing. They indicate that cholesterol was removed from the tissues in colloidal form by reticulo-endothelial cells, and became converted into droplets within the cells. These droplets differed from the vacuoles which stained with neutral red, and were probably esters of cholesterol. They were slowly absorbed, concomitant with transformation of the vacuoles from large, refractive, to very fine ones. These changes were paralleled, at slightly later intervals, by gradual increase of sudanophilic, and less regularly, osmophilic deposits within the cells. They reached a peak at 3–4 days and disappeared with 8–10 days.

The mid-dorsal region of the female sacrum.[1] Mildred TROTTER and Gordon S. LETTERMAN, Departments of Anatomy and Surgery, Washington University.

The anatomical conformation and the more common structural modifications of the female sacrum in its mid-dorsal region have been studied in a series of 674 White and Negro bones. Of these, 328 form a part of the Terry Anatomical Collection (Washington University) and 346 a part of the Todd Anatomical Collection (Western Reserve University). The sacral hiatus viewed from its dorsal aspect presents a somewhat triangular outline: the mean width of the base is 16 mm.; the length from the base to the apex has a mean of 19 mm.; and the antero-posterior diameter of the sacral canal at the level of the apex of the hiatus has a mean of 5.3 mm.

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[1] Aided by a grant from the United States Public Health Service.

The apex of the hiatus is located at the expected level, the lower third of the body of the fourth sacral vertebra, in only 35%; in 20% of the cases it lies caudad to this plane; whereas, in 45% it is found to be cephalad.

Variations in the structure of the dorsal bony wall of the sacral canal are numerous: it may be open throughout its entire length; it may be shortened either by low-lying laminae or a deficiency in development and subsequent union of the laminae and spines of one or more adjacent vertebrae; or it may have unilateral, bilateral or mid-line apertures.

A quantitative study of the seventh cranial nerve. Charles VAN BUSKIRK, Department of Anatomy, University of Minnesota. (Introduced by A. T. RASMUSSEN.)

Using normal human material obtained from 33 individuals, fiber counts of nuclei and ganglia have been utilized to determine the fiber content of the various functional components of the facial nerve. The sensory and general visceral motor fibers leave the brain dorsal to the special visceral motor fibers and do not join with the latter until just proximal to the geniculate ganglion. There was also observed in this region a mixing of facial and vestibular fibers. Cell counts indicate that the classical facial nucleus contains from 4620 to 9460 cells and that the geniculate ganglion posseses from 1462 to 3682 cells. Comparative numerical analysis of this material indicates that the human facial nerve is composed of 61% special visceral efferent axons, 21% general visceral efferent and 18% sensory fibers. The cell and fiber content of the various components of the seventh cranial nerve apparently cannot be correlated with age, sex or laterality.

The distribution of nerve cells and fibers to the gall bladder of the cat. Charles VAN BUSKIRK and Edward A. BOYDEN, Department of Anatomy, University of Minnesota.

By means of serial sections of the cystic duct, neck and body of the gall bladder, stained with the Bodian technique, it has been possible to segregate the nerves to the gall bladder and to analyze their components in the same animals in which the pathway to the sphincter of Oddi was being studied (cf. Boyden and Van Buskirk). Similarly, it was observed that 2 to 3 weeks after section of both vagi and peripheral branches of both celiac ganglia, nerve fibers were virtually eliminated from the cystic nerves. From experiments in which both vagi alone or peripheral branches of both celiac ganglia were interrupted it is clear that sympathetic components greatly outnumber the vagal components. Fiber counts will be reported at a later date. No myelinated nerves were found. In the wall of the gall bladder ganglionated nets were observed in perimuscular, muscular and mucosal layers. In the deepest layer the nerve cells were least numerous. Clusters of ganglion cells were found in the connective tissue between the liver and the convoluted portion of the duct in the region where the duct passes into the neck of the gall bladder, but elsewhere along the ducts ganglia occurred sparsely. Considering the extrahepatic ducts as a whole the point of greatest concentration of ganglia was found to be in the zone where the common bile duct enters the intestine.

Thyroid tumors in aged rats. John H. VAN DYKE, Department of Anatomy, Washington University.

Despite the view that true thyroid neoplasms, in the rat at least, are rare, examination of sixteen aged rats (801–906 days old; 10 females and 6 males) revealed nine individual thyroid cystadenomata with frequent papillary design. Eight of these occurred in seven females; one in a male. The thyroid parenchyma in each rat possessing a tumor exhibited variable but distinct features of senile atrophy which has been interpreted as histological evidence of hypothyroidism in aged rats. With only one exception these 'new growths' were accompanied by, or attached to, thin-walled cysts lined by stratified squamous epithelium; from which the basal cells appeared to have been stripped.

The evidence strongly suggests that these cysts, which invariably develop as the result of vitamin A deficiency, arise (by metaplasia) from thyroid-like follicles originally derived from ultimobranchial tissue, and that they characterize atrophic thyroid glands of old stock rats. It is also suggested that certain densely staining gland-like cells with pycnotic nuclei, some of which are 'fetal-like', arising probably from the more superficial cells of the original cyst epithelium (those undergoing degeneration), may occasionally produce densely staining, highly vascular and apparently compensating adenomata in the atrophic gland of very old rats. The origin, fate and significance of ultimobranchial cysts, 'fetal'-like cells and thyroid cystadenomata will be considered in a subsequent publication.

The thyroid gland of choline deficient mice. John H. VAN DYKE, Department of Anatomy, Washington University.

Thyroid parenchyma in young adult mice fed choline deficient diet invariably undergoes atrophy, usually within 7 to 10 days. Thyroid follicles become thin-walled, variable in size and irregular in shape. Their colloid is retracted from follicular epithelium, exhibits little evidence of resorption and may contain basophilic globules. Cytoplasm of stromal cells usually contains droplets of neutral fat, as in the parenchyma of the liver.

The center of both lobes of the thyroid is characterized by large irregularly shaped cysts lined by tall, simple columnar epithelium; certain cells of which possess a cuticular border with long tufts of cilia. Their secretion, apparently undergoing absorption, may resemble typical colloid, but is usually composed of an achromatic 'foam-like' substance that frequently contains basophilic masses.

The evidence has been interpreted as indicating that these cysts arise (by metaplasia) from certain thyroid-like follicles originally derived from the ultimobranchial body which does not possess inherent thyroid forming potencies, that during embryonic development this 'body' becomes induced by differentiating thyroid parenchyma to transform into thyroid-like tissue that may undergo metaplasia after birth in response to parenchymal atrophy; and that after choline deficiency such tissue may revert to more primitive (?), mucous-like, epithelia, characteristic of the unincorporated ultimobranchial body, which in intimate association with thyroid parenchyma may be responsible for colloid basophilism and possibly altered thyroid function.

Motion picture of the behavior of lymphatic vessels in the living bat. Richard L. WEBB and Paul A. NICOLL, Department of Anatomy, University of Illinois and Department of Physiology, Indiana University.

Three divisions of the lymphatic channels in the bat are recognized. In the larger transporting lymphatics the structure and activity are organized on a segmental basis. The second pattern of vessels evolves from the transporting channels to the peripheral area. The segmental structure of the transporting lymphatics is replaced by a thick-walled, expanded sinus representing a type of "lymph heart," while the remaining portion serves as a thin-walled collecting lymphatic. The third division is represented by large bulbous capillary structures. Their walls are so thin that they are indistinguishable from the surrounding tissue except for their remarkable positive contractions. They begin as large sacs and are connected to collecting lymphatics one or a series of bulbous structures.

The lymphatic system of the bat is characterized by its marked intrinsic activity. The nature of the activity differs with the type of vessel studied. The rate of rhythmic contraction of the bulbous capillaries is the most rapid, and the entire bulb participates in the movement. The rhythmic activity of the collecting vessels is confined chiefly to the valvular areas. In the transporting lymphatics, the movement is segmental. Each segment is confined to the region between two adjacent valves. The rhythmical contraction of the lymphatics is universal in the bat, for they may be observed in the wing, in the body wall, and in the mesenteries.

The morphogenetic properties of frozen-dried tissues.[1] Paul WEISS, Department of Zoology, The University of Chicago.

Quick-freezing and drying leaves tissues in a more normal condition than other preservative methods. Such "devitalized" tissues remain relatively unmodified though their cells are dead. This fact opens a unique experimental approach to some basic problems of morphogenesis. It makes it possible to explore the interrelationship between cells and matrix by combining the devitalized matrix of one tissue with living cells of another. Likewise, valuable results may be expected from comparing those organizing effects of one cell group upon another which persist in devitalized but structurally undisrupted tissue with those of living and of disorganized dead tissue.

In preliminary tests, various tissues of frog tadpoles and Amblystoma larvae were frozen at cca. –150°C., dehydrated at –40°C., then rehydrated and transplanted to similar larvae, mostly into the dorsal fin. Such grafts become incorporated, resist resorption and substitution, preserve their original basic texture, and become invaded by host cells. Significantly, the cell population varies with the invaded tissue: in liver, the cells are small with irregular nuclei; in cartilage, they are large with large vesicular nuclei; in muscle, they are elongate; in bone cavities, reticular. Tht invasion cells thus imitate characteristics of the native cell population.

Devitalized cartilage of Amblystoma has induced some new cartilage formation on its surface (5 months p. op.) when transplanted to the foot, but not in the dorsal fin.

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[1] Work done under contract with the Office of Scientific Research and Development.

Damming of axoplasm in constricted nerve: a sign of perpetual growth in nerve fibers.[1] Paul WEISS, Department of Zoology, University of Chicago.

The following phenomenon of "axon damming" was observed constantly in several thousand nerve fibers of rat, rabbit and chicken nerves subject to chronic local constriction. Just proximal to the constriction, the axons become greatly distended and often twisted into corkscrew shape. Farther proximally, they are beaded, with the average number of swellings per millimeter declining with increasing distance from the constriction. Axon damming is confined to that part of the fiber lying between central cell body and the near end of the constricted zone. Occasional swellings at the far side seem to belong to recurrent fibers of regenerated nerves.

Typical axon damming appears within 1 week after the constriction is applied, and continues as long as the constriction lasts; it was still observed undiminished after 8 months. After releasing a nerve from chronic constriction, the swellings spread centrifugally and the axon diameter may become equalized within a matter of days.

Mechanical considerations and model experiments, reproducing the phenomenon, suggest that the observed damming and coiling in front of a throttling "bottleneck" result from the piling up of growing axon substance pressing distad. Mature axons thus seem to grow perpetually from their central cells, undergoing commensurate peripheral dissipation, which latter phase we may tentatively identify with the discharge of substances (e.g., acetylcholin and other "neurohumors") from peripheral nerve.

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[1] Work done under contract with the Office of Scientific Research and Development.

Transplantation of frozen-dried cornea in the rat.[1] Paul WEISS and A. Cecil TAYLOR, Department of Zoology, The University of Chicago.

The good results obtained with quick-frozen, dehydrated, stored and later rehydrated nerves as grafts (Weiss and Taylor, '43, Weiss, '43) have encouraged extension of the experiments to other tissues. The superiority of freezing-drying over other preservative treatments lies in its much less radical effects on the biophysical and biochemical (enzyme, protein, etc.) constitution of the tissue. The host body may incorporate such grafts without either foreign body reaction or substitution.

Preliminary tests with cornea gave promising results. Rat corneae were frozen in Isopentane at cca. –150°C., evacuated at –40°C. for several days, then sealed and stored. Before use they were rehydrated in Ringer's in vacuo, resuming normal consistency and appearance. They were grafted over host eyes whose corneae had been excised except for a narrow rim. The exposed grafts easily succumb to postoperative infection. Uninfected grafts, however, become incorporated by fusing circumferentially with the residual rim of the host cornea. They retained their laminated structure, became resettled by host cells (in supranormal numbers), and remained transparent up to 6 weeks, when the experiments were terminated. Innervation had also returned, as the cornea (wink) reflex could be evoked from the grafts. Although microscopic examination revealed no sign of substitution of the graft matrix by the host, the permanency of the incorporation remains to be demonstrated.

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[1] Work done under contract with the Office of Scientific Research and Development.

Descensus testiculorum: descent after severance of gubernaculum. L. J. WELLS, Department of Anatomy, University of Minnesota.

Laparotomy was performed upon 87 prepuberal ground squirrels (Citellus). Gubernaculum and mesentery of epididymis and ductus deferens were unilaterally severed. Testis, being then attached only by tubular structures, was elevated without stretching to highest position between abdominal viscera. Several months later, subsequent to sexual maturity, animals were autopsied.

Forty-nine animals exhibited no adhesions which could have affected descent. In 76% of these, the testis descended on the operated side and its weight resembled that of the contralateral control. In 24%, testis was found at autopsy in the abdomen. It was about one-third as large as its control and presented histological characteristics of cryptorchidism. Nine of the 49 animals showed reversal of cranial and caudal ends of the testis on the side of severance.

During the final stage of descent in this species, the observations indicate that the gubernaculum is not requisite for descent and suggest that its chief function is to steer the testis into the inguinal bursa.

During earlier stages, when the gubernaculum occupies that portion of the abdominal wall which everts to produce the bursa and when it directs the wider epididymis into the orifice of the bursa, it is possible that the gubernaculum and associated epididymis may provide a mechanical factor which acting with androgen causes the bursa to become large enough to receive the testis (see following abstract).

Descensus testiculorum: androgenic stimulation of the scrotum, inguinal bursa and ductus deferens. L. J. WELLS, Department of Anatomy, University of Minnesota.

Data were obtained from 177 ground squirrels. About half of these animals received injections of androgen or of gonadotropins which stimulate the testis to secrete androgen. Injections caused the scrotum to become pendulous and pigmented and its hair to become coarser and longer. The inguinal bursa (processus vaginalis and surrounding layers) of injected animals was observed to be two or three times heavier than that of controls. Upon depriving the bursa of its gubernaculum, epididymis and testis, androgen still increased the weight of the bursa but less than before. The ductus deferens of injected animals was about 37 to 54% longer than that of controls.

Preliminary experiments in rats in which the gubernaculum was severed at birth supply additional and confirmatory data concerning the bursa.

The observations suggest that in Citellus and perhaps other mammals descensus testiculorum is due in part to the action of androgen upon the scrotum, inguinal bursa and ductus deferens. According to this view, androgen promotes descent by causing the bursa to become large enough to receive the testis, the ductus deferens long enough to permit descent and the scrotum large enough to receive the bursa and its contents. This is supplemented by the gubernaculum which acting as a mechanical factor assists androgen in expanding the bursa (see preceding abstract).

The size of the intestine, and the size and number of lymph nodules under certain dietary conditions. Jay L. WIERDA, Department of Anatomy, University of Pennsylvania.

Five different diets were fed to five different groups of rats for 8 weeks. The diets were (1) a balanced concentrated, (2) an agar supplemented, (3) a liver supplemented, (4) a fat-free, and (5) a carbohydrate-free, vitamin B-deficient. Six animals formed each group. The diets were fed ad libitum, beginning at weaning. The intestine was examined for lymph nodules, using a binocular microscope; a camera lucida was utilized in determining the area.

It was found that the five diets have the following descending effect in increasing the length of the small intestine: Diet 3 > Diet 2 > Diet 4 > Diet 1 > Diet 5. For the large intestine the following order of effectiveness in increasing the length was found: Diet 2 > Diet 3 > Diet 1 and 4, > Diet 5. The area of the small and large intestine follows the pattern of the order shown above. When the diets are arranged in order of increase in number of nodules observed in the wall of the small intestine, the order is: Diet 1 and Diet 2 > Diet 3 > Diet 4 > Diet 5. However, when the diets are arranged in order according to the total area of the nodules the following sequence occurs: Diet 1 > Diet 2 and Diet 3, > Diet 4 > Diet 5.

Microdissection and injection studies of thyroid follicles. Roy G. WILLIAMS, Department of Anatomy, University of Pennsylvania.

Immersion of excised thyroid follicles in water causes the cells to swell but injection of water into the intact lumina of excised follicles or of follicles in mice with the blood circulating causes the opposite effect.

Experimental hemorrhage into individual living follicles indicates that capillary pressure is greater than colloid pressure and that colloid is not sufficiently different from blood serum to cause any visible changes in erythrocytes.

The staining of the boundaries where cells meet in this gland is a post-vital phenomenon.

Small holes, defects or channels in the epithelial wall play no significant part in the diminution of colloid.

Carbon granules injected into colloid-containing follicles of surviving rats are eventually removed by macrophages and before removal provide no evidence after activation of the gland that there are channels through or between the cells or that colloid is reduced by pseudopodial action of the thyroid cells.

The effects of Suramin (Germanin) on normal and neoplastic lymphoid tissue of the mouse. W. Lane WILLIAMS, Departments of Anatomy, Yale University and Louisiana State University.

Forty C_3H mice received subcutaneous transplants of an estrogen-induced mediastinal lymphosarcoma and were killed for study 21 days later. Twenty of these received daily subcutaneous injections of 0.1 cc. of aqueous (100 mg. in 20 cc.) Suramin[1] (a non-arsenical trypanosomicide) during the last 6 to 12 days of this period. At autopsy the weights of tumors in treated animals ranged from 1 to 7 gm. (av. 3.42 gm.); controls, 7 to 16 gm. (av. 10.7 gm.).

The inhibition of tumor growth resulted from destruction of neoplastic lymphocytes. Large numbers of these necrotic cells as well as macrophages containing remnants of them were present in the tumors.

Suramin also affected the lymphoid tissue of the lymph nodes and spleen. Grossly these organs showed no significant alterations. Histologically there was a decided diminution in the number of adult lymphocytes. The usual lymphoid areas consisted almost entirely of reticular cells, large lymphocytes and plasma cells; there were few if any necrotic lymphocytes. The picture was one of depletion in the usual lymphoid tissues in contrast to the actual necrosis which was evident in the tumors.

Suramin in the dose used was ''toxic'' not only to normal and neoplastic lymphoid tissue but also caused some damage to the parenchyma of the kidneys and/or liver in approximately 20% of the animals.

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[1] ''Naphuride'' — Winthrop Chemical Company.

The effects of gastric mucin on the growth of transplanted tumor (lymphosarcoma) cells in the mouse. W. Lane WILLIAMS and Leonell C. STRONG, Departments of Anatomy, Yale University and Louisiana State University.

The purpose of this study was to determine whether or not gastric mucin inhibits the host's resistance to transplanted neoplastic cells in a fashion comparable to that by which this material favors experimental infections with microörganisms.

Sixty-nine C_3H mice were injected (subcutaneously and/or intraperitoneally) with 0.1 cc. of one or both of the following: (1) tumor tissue in normal saline; (2) tumor tissue in a solution of saline and mucin. The concentrations of tissue and mucin ranged (separately) from 1 gm. in 100 cc. to 1:400.

In animals studied from 10 to 135 days subsequent to injection, mucin prevented growth of the transplanted cells in subcutaneous sites by augmenting the usual inflammatory reactions to such a degree that the tumor cells were destroyed. These reactions took place in a very small area and usually by the twenty-fourth day there was no indication of the injection site. Hyperplasia of tumor cells attended intrapertioneal injection of the mucin-tumor mixtures in a majority of the cases (21 of 25). The mucin educed no considerable general or focal inflammatory or ''defense reaction'' in the relatively large area involved in the abdominal cavity.

In addition to those included in this report the tumor has grown successfully upon transplantation in over 200 mice. All failures have been limited to those which received tumor cells plus mucin.

Production of urinary tract calculi in albino rats by injecting estrogenic hormone. James G. WILSON and John A. BENJAMIN, Departments of Anatomy and Urology, University of Rochester. (Introduced by Karl E. MASON.)

Thirty-one male and 29 female, newborn rats were injected thrice weekly with 0.1 mg. of estradiol dipropionate during the first 4 weeks of life. Palpation and x-ray, confirmed by autopsy, revealed that stones formed in the bladder and/or urethea of 24 males, but only one female, between the second and fifteenth months of age, 83% of these appearing in the first 6 months. Untreated animals of both sexes raised under identical conditions of nutrition and caging developed no calculi.

The calculi were associated with infected urine, elevated urinary pH and obstructed urinary tract. The latter, characterized by obstruction at the site of the bulbourethral gland as indicated by dilation of the urethra proximal to this point and frequently by dilation and hypertrophy of the bladder, affords an anatomic basis for the higher incidence of lithiasis in the males. Furthermore, casts of the normal male urethra showed a narrowing of the lumen immediately distal to the junction of the bulbourethral glands, and preliminary studies on young treated animals before the first formation of stones revealed that this portion of the urethral lumen was often partially occluded by masses of desquamated epithelial cells from an undetermined source. Doubtless urinary tract infection also plays an important role.

By quantitative analysis the stones were found to consist largely of magnesium ammonium phosphate ($Mg\ NH_4PO_4 \cdot 6H_2O$).

Structural alterations in the brain in and after experimental concussion. W. F. WINDLE, R. A. GROAT and C. A. FOX, Institute of Neurology, Northwestern University.

Concussions, usually without fractures or hemorrhages were produced in guinea pigs and monkeys, and followed at intervals up to 32 days by controlled histologic studies. Immediate changes, demonstrated by perfusing with formalin a few seconds after striking the blow, were fragmentation, agglutination and disorganization of Nissl-body patterns. Chromatolysis, began within 24 hours and became marked by 6 days in large neurons of red, lateral vestibular, trigeminal spinal tract nuclei and reticular formation. Other nuclei affected by very strong concussions were cochlear, cerebellar, superior olivary and lateral reticular. The chromatolysis led to permanent cell loss. Primary motor and sensory cells were practically unaffected. In the monkey's cerebral cortex, large pyramidal cells showed structural changes but did not degenerate. Elsewhere there appeared to be no cell destruction. Extent and amount of damage in the brain stem varied with severity of concussion. Several less severe blows caused as much change as one strong blow. Nerve fibers suffered no demonstrable primary damage. Experiments were thoroughly controlled. *(The work described in this paper was done under a contract, recommended by the Committee on Medical Research between the Office of Scientific Research and Development and Northwestern University.*

Action of progesterone on the structure of the anterior hypophyses of castrated immature female rats. J. M. WOLFE, Departments of Anatomy and Pathology, Albany Medical College, Union University.

Nineteen immature female rats were castrated; beginning on the same day they received injections of from .5 to 10 mgs. of progesterone daily for 15 days. They were sacrificed 24 hours later and their anterior hypophyses were examined histologically. Fourteen untreated sisters, castrated for 15 days, served as controls. In the glands of the controls the granular basophiles were increased in number (average percentage of 15.9) and size. Signet-ring castration cells were absent but many granular basophiles contained small multiple vacuoles. Progesterone in sufficient amounts restricted to varying degrees the quantitative and cytological castration changes in the basophiles. One-half mg. daily was ineffective. One mg. daily was slightly effective in some rats and 3 mgs. or more daily was regularly effective in partially preventing the castration reaction, increasingly larger dosages being increasigly effective. However, 10 mgs. daily did not prevent a slight increase in the size and number of the granular basophiles. Three mgs. or more of progesterone daily generally restricted the degree of hypertrophy of the basophiles, and cells with small multiple vacuoles were absent. Five and 10 mgs. daily, respectively, administered to 2 immature rats over a 30-day castration period, prevented the appearance of signet-ring castration cells (which were present in the glands of 30-day castrate controls) but the granular basophiles were slightly increased in size and number.

The anatomical bases of concussion shock (blast syndrome). M. Wharton YOUNG, Department of Anatomy, Howard University.

No satisfactory anatomical bases have been established to explain the mechanism of deaths or injuries by explosive blasts. The syndrome presents absence of external trauma accompanied by hemorrhages and lacerations in the brain and lungs.

The central nervous system, inclosed in its bony incasement, and immersed in cerebrospinal fluid, presents "the mechanics of a closed box" (Guild, '27). Venous blood within the cranial cavity is separated from the cerebrospinal fluid by relatively thin elastic membranes, and drains via the jugular bulb and veins into the middle mediastinum.

Sudden increases in atmospheric pressure as observed in concussion waves increase the pressure on the outer body wall as well as the intrapulmonary, intrathoracic and mediastinal pressures (exaggerated sneeze). This reverses the venous flow into the cranial cavity and the noncompressible body fluids transmit the total force of the atmospheric pressure wave to the central nervous system multiplied in accordance with Pascal's Hydrostatic Law. This sudden blow to the nervous system simulates ordinary traumatic concussion. The enormous forces in action may produce lacerations in the brain and lungs. The increased vascular pressure may result in petechial hemorrhages or frank rupture of alveolar vessels.

Physiological experiments on dogs confirm the above thesis; viz, increased intrapulmonary pressure causes increased venous and cerebrospinal fluid pressures which are further exaggerated by compressing the thoracic and abdominal walls. Aquatic concussion is discussed.

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